EVALUATION OF THE DOMESTICATION STATUS OF COW COCKLE (*Vaccaria hispanica* [P. Mill.] Rauschert) POPULATIONS

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By

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ABSTRACT

Cow cockle (*Vaccaria hispanica* [P. Mill.] Rauschert) is a summer annual species introduced to North America from Europe. It has been investigated as a potential crop for the Canadian prairies because of its ultra–fine starch, cyclo–peptides, and saponins. However, cow cockle has a long history of being a weed in Canada and may need additional scrutiny of its weediness potential before initiating commercial production. In addition, cultivating poorly domesticated species may lead to further environmental and weed management risks; hence, an understanding of the domestication status is required. The objectives of this research were to evaluate available cow cockle germplasm i) to identify populations that are best adapted to cultivation as well as the traits responsible for such adaptation, ii) to determine seed dormancy levels in cow cockle populations and to determine how temperature and light affect seed dormancy and germination, and iii) to determine whether cow cockle populations are persistent and form a seed bank. A total of 15 cultivated, weedy, and wild cow cockle populations from different parts of the world were compared for agro–morphological, seed dormancy and seed persistence characters from 2009 to 2011. In the field persistence study, two populations including weedy (Scott weedy) and cultivated (Scott) lines were included. Cluster analysis revealed three main groups among the populations based on the traits studied. Physiological maturity, seed size, plant height and seed yield differed most among populations. The cultivated populations, Pink Beauty, Turkey, PB–87, Scott and a weedy population, UMan–89 had higher seed yield, larger seeds, and greater biomass compared to the other populations. Although weedy populations showed some adaptation to cultivation, characters relating to plant architecture, seed size and yield suggested a weedy habit. Freshly matured seeds of all the populations showed high levels of primary conditional dormancy except “Mongolia”. At optimum temperature conditions for germination (10 C), the effect of temperature regime (alternating and constant) and light on seed dormancy were insignificant. The variation in optimum temperature, light, and their interactions among the cow cockle populations may be due to the plants evolving to adapt to their local environments. In the field persistence study, the weedy population had higher seedling emergence at two out of three locations and a larger residual seed bank at all the locations. Despite the differences in seed

persistence between the populations, considerable numbers of seed of both weedy and cultivated lines were recovered from the soil seed bank at the end of the study. This concurs with the results of the laboratory persistence study, as both the populations had greater seed longevity (p_{50} values > 50 days) which suggests a field persistence of over three years. In conclusion, higher seed yield, larger seeds, and greater biomass in cultivated populations may result from certain pre–adaptation towards domestication, which may have been acquired during the process of pre–domestication cultivation. From a domestication perspective, if cow cockle were grown as a crop, the conditional dormancy may not be considered a barrier to domestication and can be viewed as a physiological mechanism to avoid germination at harvest. The major concern in cow cockle domestication would be seed persistence, as it can form a reasonably long–term seed bank. This may pose some concerns for the production of cow cockle as a crop in the Canadian Prairies. The current research suggests that cow cockle populations from Canada, although they showed some adaptation to cultivation; are largely weedy and can be considered as variants of an early introduced species which might have evolved to adapt to non–native conditions.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2, 4 – D	2, 4 – dicholorophenoxyacetic acid
AIC	Akaike information criterion
ANOVA	Analysis of variance
BM	Biomass
DR	Disease resistance
DTF	Days to flower
EM	Emergence
FP	Flowering period
FS	Flower size
GLM	Generalized linear model
LD	Linear discriminant
LSD	Least significant difference
MCPA	2–methyl, 4–chlorophenoxyacetic acid
MPa	Megapascal
PH	Plant height
PM	Physiological maturity
RCBD	Randomized complete block design
RH	Relative humidity
SAS	Statistical analysis system
SS	Seed shattering
TSW	Thousand seed weight
YL	Yield

1.0 INTRODUCTION

Vaccaria hispanica [P. Mill.] Rauschert, cow cockle, is an annual herb belonging to the Caryophyllaceae (carnation family). It is native of Eurasia and widely distributed in East Asia, South America, North America and Australia (Meesapyodsuk et al. 2007). Cow cockle is a common annual weed in many countries (Holm et al. 1997). Cultivated populations of cow cockle have been utilized for pasture (Georgia et al. 1933), as an ornamental (Chater 1964) and medicinal plant (Kumar et al. 2011; Feng 2012). In Asia, the plant is used as a medicinal herb and the seeds are referred as wang bu liu xing in traditional Chinese medicine. It has been used to activate blood circulation, to promote milk secretion, menstrual discharge and diuresis, in the treatment of amenorrhea, mastitis and also as an anti–cancer agent (Sang et al. 1999; Shengmin et al. 2000; Balsevich et al. 2012). In North America cow cockle is listed as being toxic to livestock (Balsevich et al. 2012), as it produces saponins. All the plant parts produce saponins; however, the concentration is highest in seeds.

In North America, cow cockle is an introduced species and a summer annual weed in grain fields (Balsevich 2008). In Canada, cow cockle was one among the common weeds of prairies based on field surveys from 1890s, 1940s and 1960s (Alex 1982). Competition studies in wheat and flax revealed that cow cockle has ability to cause significant yield reductions, especially with high seeding rates (Alex 1966, 1970). Cow cockle populations resistant to synthetic auxins, 2, 4 – D (2, 4 – dicholorophenoxyacetic acid), MCPA (2–methyl, 4–chlorophenoxyacetic acid) and group B/2 herbicides were also reported (Molberg 1966; Beckie 2012).

Cow cockle was one of several plants screened in Montana as a potential oil–seed species for the northern Great Plains in 1960's (Goering et al. 1966). However, cow cockle seed was found to have low oil content and instead, high concentrations of starch (Goering et al. 1966). Cow cockle is a prolific seed producer with the seed physically resembling canola but differing in composition. The main constituents of cow cockle seed are ultra–fine starch, cyclopeptides and significant amounts of mono– and bisdesmosidic triterpene saponins (Biliaderis et al. 1993; Sang et al. 1999; Balsevich et al. 2012). Potentially lucrative seed composition and favorable agronomic characteristics created interest in domestication of cow

cockle for use as an alternate crop. Although variability has been observed for plant height, seed size and maturity among cow cockle populations, preliminary agronomic studies suggested that existing machinery can be used for its production (Goering et al. 1966). In addition, the decline in wild cow cockle populations on the prairies (Thomas et al. 2007) and availability of several herbicide options to control volunteer populations (Saskatchewan Ministry of Agriculture 2011) may favor its domestication.

Domestication is the process of adapting wild plants or animals to artificial conditions for the benefit of humans. Domestication played a key role in early agriculture and it is generally accepted that the crop plants originated from wild forms through this process. Early farmers domesticated plants of their interest, by harvesting and re–sowing them, thereby creating an environment for the plants to grow in a specific pattern (Harlan 1992). Of course, all plants did not respond similarly, some plants flourished under manmade habitats and others did not (De Wet and Harlan 1975). Plants which thrive well under artificial agro–ecosystems are either domesticates or weeds. Wild species originated from naturally disturbed areas, because of their preadaptation, were considered as easy targets of selection in human created microclimates. Preadapted species with known utility were domesticated and resulted in crops. A similar adaptive process resulted in weeds, which were viewed as useless (Harlan and De Wet 1965). The basic difference between the two plant forms lies in their degree of dependence on man (De Wet and Harlan 1975). Unlike weeds, domesticates lose their self–sustaining traits such as seed dispersal, and dormancy thereby relying on humans for their survival and propagation. However, several old world crops have weedy origins, and are termed secondary crops (Vavilov 1926). Oat (*Avena sativa*), rye (*Secale cereale*) and camelina (*Camelina sativa* [L.] Crantz.) are examples of such crops. The identification of beneficial products of a weed species followed by deliberate planting and harvesting, facilitates transformation of these weeds into a crop.

Domestication involves transformation of wild phenotypes to domesticates under human cultivation. Plant traits that are modified during domestication to increase the adaptability to human intervention are collectively called the domestication syndrome (Hammer 1984; Harlan 1992). The morphological characters of a species which are selected during early domestication are important, as most of them are related to its survival and adaptation in the wild. The two key modifications in domesticated seed crops were the loss of natural seed dispersal and loss of seed

dormancy (Zohary 1969; Harlan et al. 1973; Fuller 2007). Apart from the development of tough rachis, early cereals were selected for shorter plants to avoid lodging, large spikes (and grains) and disease resistance during the process of domestication. Several studies have demonstrated the use of the morpho–physiological variability to document the transition from wild to domesticated types (Schwanitz 1957; Piperno and Pearsall 1998). The differences in these plant traits involved in the domestication process may vary within species and between species as well. Therefore, comparative evaluation of the morphology of different populations may be helpful to understand the domestication process (Harlan 1965; Schwanitz 1966).

Loss of seed dormancy was one of the first characteristics selected for in the process of domestication (Gepts 2002). Dormancy is an adaptive trait of survival for most wild and weed plants, as it ensures continuation of the species over time. In domesticates, dormancy is selectively disadvantageous, as dormant seeds do not emerge the season they are planted, resulting in uneven plant stands and volunteers in subsequent crops (Evans 1993). Maass (2006) documented a wide range of germination rates in *Lablab purpureus* with rapid germination in most cultivated populations. In addition to legumes, cereals such as rice were also reported to have lost their wild type germination inhibition during domestication (Sweeney et al. 2007). Therefore, seed dormancy is considered an important characteristic to understand the process of domestication in several species. However, studying the change in seed dormancy during the process of evolution is difficult as there are very few differences in morphology between dormant and non–dormant seeds.

Preliminary studies have indicated the presence of significant primary seed dormancy in cow cockle (Redlick unpublished). Germination of fresh cow cockle seed is often low to negligible but increases with GA_3 treatment (Hsiao 1979), suggesting that primary dormancy in cow cockle is most likely non–deep physiological dormancy. Seeds with non-deep physiological dormancy cannot germinate or will only germinate in a narrow range of temperatures (Baskin and Baskin 2004). Seeds with deep physiological dormancy possess greater limitations to germination compared to non-deep physiological dormant seeds and they are often insensitive to GA_3 treatment. Freshly harvested cow cockle seeds have been observed to germinate better in the field than in the laboratory, perhaps due to fluctuations in soil temperature. Apart from these preliminary studies, no real efforts have been made to study the seed dormancy and germination

ecology in cow cockle; knowledge of these aspects is important to understand the adaptability of cow cockle populations to cultivation.

Persistent seeds are the seeds that maintain viability for longer periods (e.g. more than one season) in the soil (soil seed bank) (Fenner and Thompson 2005). Seed persistence is another important character which generally separates domesticates from wild species, as most wild species have seed dormancy and develop persistent seed banks (Holm et al. 1997). In wild species, seed persistence aids in self–dependence, as it enhances population growth rates and reduces the risk of extinction (Kalisz and McPeek 1993; Fisher and Mattheis 1998; Adams et al 2005). However, it is generally accepted that most cultivated species cannot persist in natural habitats (Pessel et al. 2001). Hails et al. (1997) reported low seed persistence in cultivated oilseed rape populations when compared to their wild relatives. Seed persistence under field conditions is a function of multiple interacting factors such as seed morpho–physiological traits, dormancy and defense mechanisms against predation. During the process of evolution, the change in the above traits results in shift from persistent to transient seed bank formation. For example, in most wild legumes, hard seeds due to thick seed coats contribute to high seed persistence (Lush and Evans 1980). Decline in seed persistence of cultivated populations was due to the evolution of reduced seed coat thickness during the process of domestication.

Phenotypic modifications, loss of seed dormancy and reduced seed persistence are some of the important characteristics a wild or weedy species should acquire in the process of domestication to adapt to the conditions of cultivation. Considering these important characteristics of the domestication syndrome, the focus of my thesis research was to study the domestication status of cow cockle. Because cow cockle is an introduced summer annual I hypothesize different populations will exhibit varying degrees of weediness. To test this hypothesis, three studies were conducted to investigate the different components of the domestication syndrome. The objectives of this research were to evaluate available cow cockle germplasm,

1. To identify the populations that are best adapted to cultivation as well as the traits responsible for such adaptation.

2. To determine seed dormancy in 15 cow cockle populations and to determine how temperature and light affect seed dormancy and germination.

3. To determine whether cow cockle populations are persistent and form a seed bank.

The study on domestication status of cow cockle is required to understand the traits with adaptive significance during the process of domestication. Evaluating different populations for agronomic and morphological traits will provide information on its potential as a secondary crop for the Canadian Prairies, and may be of interest to breeders and producers. Studies on seed dormancy and persistence among the cow cockle populations will enable us to understand the propensity for cow cockle to be weedy in agricultural situations. Furthermore, the variability among these populations is expected to be useful for genetic improvement and further breeding programs.

2.0 REVIEW OF LITERATURE

2.1 Cow cockle

2.1.1 Distribution and Taxonomy

Cow cockle also known as cowherb, China cockle, spring cockle or soapwort, was introduced to North America as an ornamental plant from Eurasia (Frankton and Mulligan 1987). In Europe, it was grown as a forage plant, where the genus, Vaccaria, is named after its potential as cow feed (Georgia et al. 1933; Goering et al. 1966). It is an annual herb in the Caryophyllaceae, once a common weed in grain fields and abandoned plots (Chater 1964). Now it is considered as a minor weed in fine textured soils of the United States and the Canadian Prairies. The species has a wide distributional range as a native species including northern Africa, temperate Asia and most parts of Europe **(**USDA, ARS, National Genetic Resources Program). Furthermore, the species was reported to have been naturalized in many parts of South Africa, temperate and tropical Asia, Australia, North and South America (Elias 2006). From a taxonomical point of view, *Vaccaria* is a monotypic genus with a single taxon *Vaccaria hispanica* (P. Mill.) Rauschert (Ross 1966). However, due to variable intra–specific taxonomic treatment, several synonyms are under use in the literature including *Saponaria vaccaria* L., *Vaccaria parviflora* Moench., *Vaccaria pyramidata* Medik. and *Vaccaria segetalis* Garcke (Thieret and Rabeler 2005).

Cow cockle is erect and branched, grows to a height of 20–100 cm and has a tap root system (Fletcher 1897). The stem is smooth and succulent throughout. Leaves are opposite ovate–lanceolate and clasping around the stem. The inflorescence consists of flowers in loose cymes with 5 petals (typically of pink color) and the calyx ridged with 5 sepals forming a tube. Capsules are enclosed in a calyx tube, produce 10–20 rough black, globose to sub–globose seeds with a diploid chromosomal number (2n) of 30 (Talavera 1978). The species is propagated through seed, which germinates predominantly during spring from late April to late May and flowers in July. Cow cockle seeds continue to germinate throughout the summer but in reduced numbers. It starts flowering in July and at maturity, the apex of the capsule opens into a four–toothed orifice to disperse the seed during the fall. At maturity, the plant tops break off to form tumbleweed carrying their seed with them to considerable distances (Crandall 1893).

The seeds contain saponins which are poisonous upon ingestion to livestock. Saponins are known to cause a violent gastro–enteritis with vomiting, diarrhea and colic**.** Absorption by bloodstream causes breakdown of red blood cells and injury to the central nervous system producing convulsions and paralysis (Ministry of Agriculture Food and Rural Affairs, Ontario). However, some studies revealed positive effects of saponins especially in ruminants (Makkar and Becker 1996). Although toxic to livestock, they are harmless to humans as they are poorly absorbed by the digestive system.

2.1.2 Cow cockle as a weed

Cow cockle (*Vaccaria hispanica* [P. Mill.] Rauschert) is one of the many weed species that was introduced to North America from Europe (Chestnut and Wilcox 1901). In North America, cow cockle was initially introduced for ornament as early as 1874 (Crandall 1893). In Canada, the first report of cow cockle was from Manitoba and later it spread to north–western provinces (Fletcher 1897; Crandall 1893). The rapid spread of cow cockle in the Prairie Provinces resulted in listing this species as a "Noxious Weed" (Alex 1982). Although occurrence of *Vaccaria* in Western Canada dated to the mid–19th century, it became widely distributed, especially under fine textured soils by 1960 (Alex 1966). At this time, densities in some fields exceed 300 plants m^{-2} (Alex 1968).

The early emerging cohorts of cow cockle in spring with their profusely branching head interfere with the light interception of grain crops. Yield reductions in crops such as wheat and flax have been reported in several studies (Alex 1968, 1970; Tanji and Zimdahl 1997). Greenhouse studies by Rahali (1982) revealed that the wheat shoot dry weight varied from 4.4 to 1.7 g $plant^{-1}$ at 252 plants m^{-2}, when cow cockle was grown at 21 and 168 plants m^{-2}.

Some of the early management techniques to control cow cockle included hand pulling, early summer fallowing at short regular intervals, thorough cleaning of seed, deep sowing and in–crop harrowing (Fletcher 1897). Cow cockle populations resistant to synthetic auxins and commonly used broad–leaf weed herbicides MCPA (2–methyl, 4–chlorophenoxyacetic acid) and 2, 4–D (2, 4 – dicholorophenoxyacetic acid) and group B/2 herbicides have also been reported (Molberg 1966; Beckie 2012). However, a decrease in its frequency of occurrence has been observed in Western Canada recently (Thomas et al. 2007). Weed management practices such as

herbicides and zero tillage were attributed for this reduction in weed populations (Harker et al. 2009). Similar reports were also obtained from Central Europe, where the advent of new methods of seed cleaning, application of chemical weed control and improving agricultural engineering in the last few decades has reduced the frequency and distribution of cow cockle (Kiraly et al. 2006).

2.1.3 Utilization

Cow cockle is being considered for domestication because of its seed composition. The seed contains 60–65% starch, 11–15% protein, 3–4% oil and around 2–4% saponins (Balsevich 2008). Cow cockle produces uniform, small (0.3–1.5 µm), polygonal starch granules with gelatinizing and pasting properties, which may be of interest to the food industry (Biliaderis et al. 1993). The unique ultra–fine starch granules of cow cockle may serve as an alternate source of carbohydrate or fat replacers (Reichert et al. 1986). The small granule size of cow cockle starch makes it suitable as dusting starch in cosmetics and industrial dusting (Goering and Brelsford 1966). Although preliminary work suggests the unusual properties of cow cockle starch, further research has largely been limited due to lack of proper extraction techniques (Sang et al. 2003). Goering et al. (1966) failed to extract considerable amounts of starch with Dimler's process and suggested a modified wet milling technique for better extraction of cow cockle starch. Proteins, specifically cyclic peptides obtained from *Vaccaria* seed have a wide range of anti–microbial properties (Efthimiadou et al. 2012). Besides the basic components, cow cockle seeds also contain bioactive components such as alkaloids, phenolic acids, flavanoids and steroids which exhibit a wide range of structure–dependent bioactivities and may have potential markets (Mazza et al. 1992; Sang et al. 2003).

Although cow cockle is an annual weed in North America, it is considered as an important component of traditional Chinese medicine. The seed is known as wang bu liu xing in Chinese medicine and used as a galactagogue, which is treatment for menstrual problems and deficiency of lactation (Sang et al. 2003). A screening study of several Chinese herbs by Shoemaker et al. (2005) identified the cytotoxic activity of aqueous extracts of *Vaccaria hispanica* seeds. Phytochemical investigations of cow cockle seeds revealed the presence of

mono– and bisdesmosidic triterpene saponins (Sang et al. 2003; Balsevich et al. 2006) similar to those found in *Quillaja saponaria* (Guo et al. 1998).

Several studies have revealed potential anti–cancer activity of *Vaccaria hispanica* seed extracts (Shrestha and Baik 2010). Increased evidence of biological activity of cow cockle saponins has prompted research into investigation on optimizing the extraction conditions (Guclu–Ustundag et al. 2007). Guclu–Ustundag et al. (2007) reported a comprehensive list of saponins isolated from cow cockle.

Attempts towards commercialization of this species have been made, targeting rapid production and adaptation of this species in Canadian prairies under the trade name of Prairie Carnation® (Saponin Inc. 2003). In the process of commercialization, a double haploidy protocol (Microspore culture) for the production of rapid homozygous populations was developed (Kernan and Ferrie 2005). However, Saponin Inc., based out of Saskatoon, SK, Canada has not been completely successful, as the company fell into receivership in 2011, but has since restarted (Willenborg and Johnson 2013).

2.2 Domestication

2.2.1 Significance of domestication

Domestication is a complex evolutionary process that leads to increased adaptation of plants to human management (Gepts 2004). The modern agriculture that we are practicing today was started by domesticating plants about 10,000 years ago (Gepts 2004). Before the advent of agriculture, our ancestors were hunter–gatherers for hundreds of thousands of years. The shift from a nomadic hunter–gatherer system to sedentary agricultural societies occurred with the aid of domesticated plants (Meyer et al. 2012). The utility and reliability of the tamed plants were the main reasons for the ready uptake of agriculture by ancient hunter–gatherers.

Early farmers domesticated plants of interest, by successive harvesting and re–sowing, thereby changing the key alleles in the genomes of cultivated populations (Fuller et al. 2010). During the domestication process, wild forms were transformed to domesticates by altering key traits (Guglielmini et al. 2007). Domesticates that contributed to agriculture were the desirable plants, which were adapted to human created microclimates.

Human intervention not only resulted in domesticates, but also weeds, which are generally considered intermediate between the wild and domesticates (Anderson 1952; Harlan et al. 1973). In most cases, the weedy populations can be clearly distinguished from wild forms morphologically (Harlan et al. 1973). For example, shattering canes of sorghum (*Sorghum bicolor* subsp. *Bicolor*), Asian weedy rice (*Oryza* sps.) and weedy races of barley (*Hordeum spontaneum* Koch) can be easily distinguishable from their wild populations (Harlan et al. 1973). Yamaguchi (1992) reported that weedy azuki (*Vigna angularis* (Willd.) Ohwi and H. Ohashi) plants share characters with both cultivated and wild populations. The weedy species possess plant architecture similar to those of the cultivars while maintaining the wild mode of seed dispersal and pod dehiscence. Most weeds evolved directly from wild plants through adapting to continuous disturbances under man–made habitat conditions (Guglielmini et al. 2007). Unlike wild plants, weeds are capable of colonizing man–made habitats without any requirement of artificial propagation (Guglielmini et al. 2007). Conversely, weeds and domesticates generally cannot compete successfully with wild forms for natural habitats (De Wet and Harlan 1975). Domesticates differ from wild and weed plants based on the degree of dependence on humans. These plants generally lost the ability to disperse seed by natural means and depend on humans for their survival and propagation (Allaby et al. 2008; Purugganan and Fuller 2011). However, wild and weed plants retain their self–dependent characters and never require artificial propagation.

Of the total 250,000 angiosperm species, it is estimated that 2500 plant species have been domesticated into primary and secondary crops (Meyer et al. 2012). Primary crops are the crops which are derived from wild progenitors, whereas secondary crops are evolved from weeds in the primary crops. This basic composite of domesticated plants increased gradually and the domestication of new wild species was less frequent in recent times (Kupzow 1980). A considerable number of old world crops have initially entered the crop assemblage through domestication of their weedy forms (Vavilov 1926). Identification of beneficial products of a weed species followed by deliberate planting facilitates transformation into a crop (Sauer 1950; Anderson 1952). According to Vavilov (1951), prior to domestication, both rye (*Secale cereale*) and oat (*Avena sativa*) were weeds, eventually entered the domestication process as useful plants. Other crops such as hemp (*Cannabis sativa*), amaranth (*Amaranthus cruentus*, *A.*

hypochondriacus and *A. caudatus*) sunflower (*Helianthus annuus* L.), false flax (*Camelina sativa* [L.] Crantz.) and common vetch (*Vicia sativa* L.) also developed from weeds directly.

2.2.2 Domestication syndrome

The set of traits that distinguishes most crop plants from their wild ancestors and relatives are commonly known as domestication syndrome (Hammer 1984; Zohary and Hopf 2000; Gepts 2004). These characters can be related to different selection pressures in terms of what causes them to evolve. From a human point of view, these are the traits most appropriate for their needs. A domestication syndrome may include combination of several different traits and observed to be different for different kinds of crop plants (Fuller 2007). In the case of cereals, loss of natural seed dispersal is often regarded as the most important domestication trait (Fuller and Allaby 2009). However, in legumes, loss of seed dormancy is thought to be more important than pod dehiscence (Ladizinsky 1979; Abbo et al. 2009). Meyer et al. (2012) observed a high diversity in suites of domestication traits in food crops. Some of the important traits of seed–propagated crops include non–shattering, loss of seed dormancy, increase in fruit or seed size, change in reproduction strategy and change in secondary metabolites (Gepts 2004). For example in cereals, a tough rachis prevents the breaking of heads and release of seeds at maturity. In contrast, abscission layer formation results in the rachis breaking at maturity in wild cereals (Gepts 2004). Similarly, delayed pod dehiscence and evolution of uniform and rapid germination (loss of physical dormancy) were key steps in legume domestication (Evans 1976).

2.2.3 Degree of domestication

The degree of domestication among cultivated plants depicts their dependence on humans for survival and propagation. In general domesticated plants differ morphologically and genetically from their wild progenitors as a result of artificial selection. For example, crops such as maize, rice, common bean and peanut are considered highly domesticated as they have lost their ability to survive under natural conditions (Meyer et al. 2012). Semi–domesticated crops are plants under cultivation but not very distinct from their wild ancestors and generally produce wide range of domesticated phenotypes (Clement 1999). However, undomesticated populations are wild plants never considered for artificial selection and can survive independently under natural environments (Gepts 2004). For instance, the cultivated cranberry plant has undergone

minor changes from its wild relative. Most cranberry varieties are essentially clones of wild species selected by early farmers (Warwick and Stewart 2005). Crops such as canola (*Brassica napus* L.) and sesame (*Sesamum indicum* L.) are considered incompletely domesticated by some scientists because of their excessive seed shattering at maturity, a wild mode of seed dispersal (Gepts 2004). Additionally, in canola, although seeds are non–dormant at maturity, the high secondary dormancy potential that contributes to persistent seed bank formation (Gulden et al. 2003) is another wild character.

2.3 Morphological variability

2.3.1 Significance of morphological variability

Domestication is a dynamic selection process (Gepts 2004), during which, plants undergo several morphological (and genetic) changes that represent adaptations to cultivation or human intervention (Parra et al. 2010). The most common morphological features of the domestication syndrome include loss of dispersal mechanisms, loss of seed dormancy (physical), changes in plant habit, gigantism and loss of mechanical protection (Whitaker 1974; Johns 1989; Casas et al. 1999). In the initial phase of domestication, these morphological differences played a key role in selection by early humans. It has been debated whether this selection was conscious or unconscious (Zohary et al. 1998). Several researchers support the role of unconscious selection for most of the morphological differences that distinguish domesticated seed crops and their wild progenitors (Darwin 1859; Darlington 1956; Heiser 1988; Zohary et al. 1998; Purugganan and Fuller 2009). However some scientists feel that early farmers were conscious in their selection process (Harlan et al. 1973; Gepts 2004; Zohary 2004, Abbo et al. 2012). In spite of the controversy, it is clear that either natural or artificial selection resulted in phenotypic changes as an adaptation to human intervention. These morphological modifications have been critical for studying the underlying genetic mechanisms during the evolutionary process (Fuller and Allaby 2009). Several comparative studies evaluating the morphology of domesticates and their wild progenitors reveal changes in plant characteristics such as seed dispersal and germination (Darwin 1859; Harlan 1965; Schwanitz 1966). In some species, these changes were so marked that plant taxonomists have often classified domesticates and their wild counterparts in different taxa (Gepts 2004). In contrast Townsend and Guest (1974) observed that wild and domesticated

grass pea populations were indistinguishable due to lack of morphological differences. Comprehensive studies have been conducted by several scientists and provided information on morphological changes associated with domestication (Schwanitz 1966; Purseglove 1968; Harlan et al. 1973; Hawkes 1983; Murray 1984).

2.3.2 Seed shattering and loss of seed dormancy

Although non–shattering is a classic domestication trait of several species (Purugganan and Fuller 2009), it is more conspicuous in cereals (Harlan et al. 1973). In wheat and barley, differences in rachis morphology were utilized to distinguish wild and domesticated populations, and documenting the transition between them (Harlan et al. 1973). In legumes, natural seed dispersal is by pod dehiscence. In domesticated legumes, this is removed or delayed due to change in pod layer morphology (Fuller and Harvey 2006). Similar trends towards non–dehiscence during domestication was also observed in flax (Zohary and Hopf 2000). However, in sesame, most domesticated forms show various degrees of pod dehiscence.

Rapid uniform germination and lack of seed dormancy is a characteristic of crops. In contrast the presence of seed dormancy is an adaptive trait in wild or weed plants. It is generally difficult to study the changes in seed dormancy in the archaeological record with domestication due to a lack of visible morphological traits related to dormancy (Fuller and Allaby 2009). Finch–Savage and Leubner–Metzger (2006) classified dormancy as morphological, physiological non–deep, physiological deep and physical. Physical dormancy or coat dormancy is due to development of impermeable seed coat and it is the most common dormancy mechanism in wild legumes (Ladizinsky 1975). The transition from wild to domesticated involves thinning of seed coats and change in other seed morphological characters (Fuller and Allaby 2009). A change in testa thickness during domestication was well documented in Andean chenopods and peas (Butler 1990; Nordstrom 1990; Eisentraut 1998; Bruno 2001).

2.3.3 Plant habit

Reduced branching or tillering and greater apical dominance is generally associated with the domestication syndrome of many crops such as maize, sunflower (Harlan et al. 1973), sorghum, pearl millet and wheat (Evans and Dustone 1970). When compared with crop plants,

wild plants are taller and indeterminate in their growth habit. This is evident in case of beans, where most domesticated beans are compact, bushy with reduced nodes, branched and have shorter internodes, whereas, wild beans are characterized by indeterminate growth and flowering (Gepts 2002). Plant growth habit has been subjected to both direct and indirect selection during the process of domestication. Unconsciously, the selection for higher yield or harvest index lead to the evolution of self–pruned varieties in tomato, by reducing lateral branching (Evans 1993). Karagoz et al. (2006) observed a noticeable variation in this trait between wild and domesticated wheat during the selection for yield. In case of cassava, an unusual variation in the type of germination was observed. The cultivated species (*Manihot esculenta Spp. esculenta*) has epigeal germination, whereas the immediate wild relative of cultivated cassava (*Manihot esculenta Spp. flabellifolia*) has hypogeal germination (Pujol et al. 2005). Other characters associated with determinate behavior include synchronization in maturity which is often observed in domesticates (Harlan et al. 1973).

2.3.4 Gigantism

Substantial increase in seed size has characterized the domestication of many cereal and legume crops (Schwanitz 1966; Hawkes 1983; Evans 1993). The variation of flower size and morphology are associated with domestication in several crops. Domesticated species possess thicker, longer and compact panicles, while in contrast; wild species are characterized by narrow and shorter panicles (Poncet et al. 1998). In the case of Brassica species, abnormal increase in flower size was observed in their wild relatives in the process of evolution (Purugganan et al. 2000). Selection for fruit size, shape and color were utilized to distinguish cultivated populations from their wild counterparts in chile peppers, potatoes and in some cucurbits (Mallick and Masui 1986; Pickersgill 2007). In cereals (Paterson et al. 1995) as well as legumes such as cowpea (*Vigna unguiculata* (L.) Walp.) and mungbean (*Vigna radiata* (L.) R.Wilczek) (Fatokun et al. 1992), crop seeds have been observed to be larger than wild seeds. Larger seed with high energy or nutrients, high vigor and competitive ability is attributed as the main reason for selection of larger seed size during domestication in several species.

2.3.5 Loss of chemical and mechanical protection

Plants produce a wide range of secondary metabolites to defend themselves against herbivores (Schoonhoven et al. 1998). These metabolites are normally lost either completely or partially during domestication, particularly in food crops (Pickersgill 2007). For example in cassava, wild populations contain high levels of cyanogenic glucoside as protection against insect pests. This is reduced in the sweet cultivars of cassava selected by humans (Gepts and Papa 2002). Similarly, the bitter and toxic quinolizidine alkaloids of wild lupins are reduced in sweet lupins which as a result are susceptible to wide range of pathogens and herbivores (Wink 1988). In potato, reduction of alkaloid levels was one of the important characters of its domestication syndrome (Johns 1989). Domestication of some species involves loss of mechanical means of protection such as spines and prickles. Cultivated *Solanum* species (for example, *Solanum quitoense*) often lack prickles or those are highly reduced when compared to their wild counterparts (Heiser 1988). Similarly, spineless cultivars of cocona (*Solanum sessiliflorum*) were evolved due to strong selection against spines during domestication (Salick 1992).

2.4 Seed dormancy

2.4.1 Significance of seed dormancy

Dormancy is the inability of a viable seed to germinate under any combination of environmental factors that are otherwise favorable for its germination, in a non–dormant seed (Baskin and Baskin 2004). Seed dormancy has been an important characteristic, which ensures distinct differentiation between domesticated and other categories (wild or weed) in the evolutionary trend. Seed dormancy is often associated with the presence of thick seed coats impermeable to water and/or presence of germination inhibitors in the testa (Pickersgill 2007). In *Chenopodium berlandieri* Moq, the loss of seed dormancy during domestication was characterized by increased seed size and thinner seed coats (Smith 1984). Seed coat dormancy is selectively disadvantageous especially in food crops, not only because it results in erratic germination. In addition, a hard seed coat may pose cooking difficulties (Lush and Evans 1980). Seed dormancy is essential for wild plants in many environments, as it maintains a soil seed bank for prolonged periods and ensures germination under most ideal conditions (Harlan et al. 1973).

Therefore a key step in the process of crop domestication is the loss of seed dormancy and evolution of uniform seed germination.

In crops, a lack of dormancy is selectively advantageous as it produces dependable germination and a predictable plant population in the field. In agriculture, due to deliberate planting and harvesting, the selection occurs against dormancy automatically (De Wet 1975). This is because the erratic germination of dormant seeds contributes less to the final crop stand (Fuller and Allaby 2009). The evolution of non–dormancy during early domestication was not spontaneous but is thought to have occurred over a period of time (Zohary 1989). This delay was may be due to fact that presence of dormancy is the dominant trait over non–dormancy and hybrids between wild and cultivated produce offspring with various degrees of dormancy (De Wet 1975). Additionally, the recent protracted domestication model (Allaby et al. 2008; Purugganan and Fuller 2011) supports the idea of plant domestication preceded by prolonged pre–domestication cultivation and in process may have resulted in non–dormant mutants. In contrast, scientists such as Ladizinsky (1987) and Abbo et al. (2011) argue that evolution of non–dormant seeds during domestication was much faster event, particularly in legumes. However, none of these hypotheses have been accepted universally. Although, seed dormancy is undesirable in domesticates, in crops such as wheat which can suffer from pre–harvest sprouting, a partial dormancy is advantageous (Biddulph et al. 2007).

Based on time of induction, there are two classes of dormancy, primary and secondary. Primary dormancy is induced during seed development. The major function of primary dormancy is to prevent precocious germination (Farnsworth 2000). Abscisic acid (ABA) is considered as an important factor in seed dormancy regulation in most species (Bewley 1997; Kucera et al. 2005). Although primary dormancy is genotypic in nature, it is also affected by the maturation environment of the mother plant. Primary dormancy is transient and declines both prior to seed dispersal and subsequently. The relief from primary dormancy after a period of dry storage is termed after–ripening (Bewley and Black 1994). The loss of primary dormancy is influenced primarily by temperature. For example in *Poa annua* L. Standifer and Wilson (1988) observed an early seed dormancy loss for seeds stored at warmer temperatures compared to seed stored at 15 C or lower.

Secondary dormancy is responsible for preventing seed germination for prolonged periods and it usually results in the formation of persistent seed banks. Secondary dormancy is induced after the release of primary dormancy due to unfavorable environmental conditions. Secondary seed dormancy acts as a selective advantage in weed species by restricting the germination of viable seed especially in areas with unfavorable seasons (Harlan 1992). This mechanism helps in maintaining the species through generations without extinction. According to Baskin and Baskin (1998), dormancy is classified into, physiological, morphological, morpho–physiological and physical dormancy. Among the various types, physiological dormancy is considered as most advanced and widespread class of dormancy. As with most summer annuals (Baskin and Baskin 1998), cow cockle exhibits physiological dormancy (Redlick unpublished, undergraduate student, University of Saskatchewan).

2.4.2 Environmental factors controlling seed dormancy

Dormancy is commonly considered as a physiologically inactive stage; however, dormant seeds are highly active in response to environmental signals (Vleeshouwers et al. 1995). Seed dormancy induction and breakage are regulated by various environmental factors such as temperature and light, however the response varies among different species (Jha et al. 2010; Karlsson and Milberg 2007).

2.4.2.1 Temperature

Temperature is regarded as the major environmental factor responsible for changes in seed dormancy under field conditions (Benech–Arnold et al. 2000). Considerable work has been done to explain the effect of temperature on the dormancy status of seeds (Baskin and Baskin 1998). Loss of dormancy is considered one of the prime characters during the process of domestication (Ladinzsky 1987). Intra–specific variability in seed dormancy associated with environmental factors such as temperature has been well studied (Leon et al. 2006). Little or no dormancy was observed in some domesticated genotypes of *Brassica napus* and *Brassica rapa* at low temperatures (Landbo and Jorgensen 1997). However, Perkun et al. (1997) reported the induction of secondary dormancy at low temperatures in the same species. This variability was explained in terms of genotypic differences in the development of secondary dormancy (Squire

et al. 1997). Genotypic background was attributed for differential germination characteristics in seeds of many populations (Young and Evans 1989).

Summer annuals, are plants whose seeds germinate in spring, grow in summer and shed seed in the fall. Due to the prevailing adverse environmental conditions at the time of dispersal, many summer annual species avoid germination immediately after shedding by various mechanisms such as primary dormancy, shading by a leaf canopy and lack of moisture (Schutz and Rave 1999). Summer annual seeds often require a low temperature exposure, known as stratification, which prevents them from germination directly after dispersal in summer (Probert 1992). This reduces high seedling mortality in these plants under relatively harsh winter conditions. Hence, cold stratification acts as an environmental cue to alleviate seed dormancy and ensure germination in spring or early summer (Probert 1992). Kruk and Benech–Arnold (1998) demonstrated that increased rates of germination after chilling treatment was due to a decline in the minimum temperature or broadening of thermal range permissive for seed germination. In contrast, high summer temperatures re–induce dormancy by increasing the temperature requirement for germination or narrowing of thermal range (Benech–Arnold et al. 1990; Bouwmeester and Karssen 1992).

2.4.2.2 Temperature Regime (Constant vs Fluctuating)

Diurnal temperature fluctuations act as a gap– or soil depth–sensing mechanism during seed germination in many species (Thompson and Grime 1983; Fenner and Thompson 2005). In addition other environmental factors such as light (Fenner and Thompson 2005) and cold stratification (Brandel 2004) can substitute the effect of temperature fluctuation. The ability of fluctuating temperatures to break dormancy compared to constant temperatures has been found to be greater in several weed species (Garcia–Huidobro et al. 1982; Probert et al. 1985; Zheng et al. 2004). In some species, fluctuating temperature is an absolute requirement for dormancy removal (Benech–Arnold et al. 1995; Baskin and Baskin 1998). In the case of *Leymus chinensis*, Hu et al. (2012) observed that temperature amplitudes >10 C fluctuation increased seed germination significantly when compared to constant temperatures. Roundy et al. (1992) also reported greater germination under fluctuating temperatures conditions compared to constant temperatures in three lehmann lovegrass species (*Eragrostis lemanniana* Nees). Fluctuating

temperatures can also overcome physical dormancy in *Cyanara cardunculus* (Huarte and Benech–Arnold 2010). Paz and Vazquez–Yanes (1998) observed differential germination patterns in wild and domesticated species of papaya under fluctuating temperatures. Although a large number of studies have demonstrated the effect of temperature fluctuation on seed germination, the physiological and biochemical mechanisms underlying this mechanism are still largely unknown (Haurte and Benech–Arnold 2010). Some studies have revealed the involvement of plant hormones in seed germination and the dormancy responses to fluctuating temperatures (Benech–Arnold et al. 1995; Huarte and Benech–Arnold 2010).

2.4.2.3 Light

Light is another important ecological cue that regulates seed germination in many species (Bewley and Black 1982). Light acts similar to temperature fluctuation, as a soil–depth indicator that prevents seed from germinating in the absence of light (Schutz et al. 2002; Cristaudo et al. 2007). The presence of light prevents germination in some plants (Morgan 1998), specifically species belonging to areas with rapid drying soil conditions. This allows the plants to avoid germination on the soil surface and subsequent desiccation (Richards and Lamont 1996). Light regulates germination mainly through the action of phytochromes (Heggie et al. 2005). It involves photo–conversion of R (red light)–absorbing phytochrome (P_r) to FR (far–red light)–absorbing phytochrome (P_{fr}) and vice versa. When R (red light) is absorbed by P_r, P_{fr} is formed and absorption of FR (far–red light) by P_{fr}, leads to formation of P_r (Kendrick and Spruit 1977). The active FR (far–red light)–absorbing phytochrome (P_{fr}) is often associated with seed dormancy breaking (Pons 1992).

The effect of light on seed germination has been well documented. For many species, exposure to light breaks dormancy and promotes germination (Bhagirath and Johnson 2008). For example, seeds of Chinese sprangletop (*Leptochloa chinensis* [L.] Nees.) required light to stimulate germination. In some species, exposure to light inhibits germination (Malik and Vanden Born 1987).

Many domesticated species are independent of light requirement for their germination when compared to seeds of wild plants (Vazques Yanes et al. 1996). This variation could be due to corresponding genetic changes in the phytochrome system during domestication. In the case of

papaya, the wild species showed an absolute requirement of light for germination, whereas domesticates were less dependent on light (Paz and Vazquez – Yanes 1998). There has been a little reported work, in understanding the change in light requirements during the process of domestication.

2.5 Seed Persistence

2.5.1 Significance of seed persistence

Seeds that reach the soil surface after dispersal may germinate, die or persist in soil (Forcella 2003). Persistence of a species is well known by its ability to form a seed bank. The seed bank is a reserve of viable seeds. When the seeds are still on the plant they form an aerial seed bank and when seeds are, on or buried in the soil, a non–aerial or soil seed bank is formed (Roberts, 1981). Most general classification categorizes soil seed banks into transient (seeds live < 1 year) and persistent (seeds live > 1 year) (Thompson and Grime 1979). Seed persistence of the soil seed bank has received greater attention, owing to its formation of long–lived seed banks which have great significance to plant population dynamics.

Domestication is considered to reduce the weedy characteristics of a species (Schlichting and Mousseau 2009). Along with natural seed dispersal and seed dormancy, seed persistence is also known to favour the self–growing abilities of weed or wild plants (Adams et al. 2005). Most wild species develop persistent seed banks (Holm 1997) when compared to domesticates, which are less persistent or develop transient seed banks. Seed persistence in domesticated crops is undesirable and can lead to problems such as volunteer weeds and ferality (Lutman et al. 2003; Gressel 2005). Vaughan (1994) observed that the wild rice seeds were significantly longer–lived compared to their domesticated counterparts. Similarly, Chadoeuf et al. (1998) demonstrated a very low seed bank survival of domesticated populations of oilseed rape in comparison with that of wild relatives. Two important experiments in seed persistence research (Beal 1879 and Duvel 1905) examined relative seed longevity of range of species including weeds. These long–term experiments include seed burial (in sand, upturned, unstoppered bottles and in earthenware pots) and sampling at regular intervals for longevity. These studies have shown significant differences in the seed longevity between crop and weed seeds (Toole and Brown 1946; Kivilaan and Bandurski 1981). Inter–population variability in seed persistence was also reported in some

species. For example, Harrison et al. (2003) found a considerable variation in seed bank formation among different populations of giant ragweed (*Ambrosia trifida* L.).

Seed persistence is a species trait that can be modified by environmental factors (Cavieries and Arroyo 2001). Although seed persistence of a species has a genetic base (Momoh et al. 2002), both morphological (for example, seed size and shape) and physiological germination traits (seed dormancy) potentially contribute to soil seed persistence (Silvertown 1999). The role of seed morpho–physiological traits that contribute to soil seed persistence has been well studied (Thompson and Grime 1983; Baskin and Baskin 1998; Thompson et al. 2003; Moles and Westoby 2006; Venable 2007). Seed morphological characters such as size and shape have been suggested to be associated to soil seed bank persistence (Thompson et al. 1993). The relationship between seed morphological characters and persistence may be because of the ease of burial. However, the correlation with seed size is not universal (Yu et al. 2007), as studies revealed both positive (Moles and Westoby 2006) and negative (Bekker et al. 1998) associations of seed size and persistence. Leishman and Westoby (1998) failed to correlate seed size and shape with persistence in 101 Australian species over a range of habitats. A possible relation between seed coat thickness and seed persistence was observed in several legume species (Ladinzinsky 1985; Bruno 2001; Gardarin et al. 2010). Physiological traits such as dormancy contribute to seed persistence by their ability to time the germination under the most favorable conditions (Thompson and Grime 1983; Donohue 2005).

The role of seed dormancy in seed bank persistence is not universally accepted (Thompson et al. 2003). Dormancy/non–dormancy cycling in most arable weed species ensures a small fraction of seeds to recruit annually from the soil seed bank (Baskin and Baskin 1998). Many ecological studies attribute dormancy as the main reason for persistent seed bank formation (Benech–Arnold et al. 2000). Cavieres and Arroyo (2001) determined that formation of persistent seed bank of a species depends on its seed dormancy. However Thompson et al. (2003) have demonstrated a weak relationship between seed persistence and seed dormancy across species and sites.

2.6 Seed persistence (Laboratory)

Seed longevity is considered as an inherent characteristic of seed that is determined by underlying genes (Bekker et al. 2003; Davis et al. 2008). Field persistence experiments are considered more reliable and informative than laboratory experiments. Inclusion of environmental factors such as soil temperature and moisture is the main reason for their wide acceptability in estimating seed persistence. However, field experiments are considered to be more tedious, much more time consuming (several years) and less economical compared to laboratory experiments (Thompson et al. 1993). Moreover, to understand the genotypic differences of seed persistence precisely, controlled laboratory studies are expected to be more appropriate than field studies, where several other factors also operate.

Of the different environmental factors, temperature and moisture content (relative humidity) are two very important factors considered for preserving seed viability (Walters et al. 2005). Temperature and relative humidity have been used in recent studies to estimate the seed persistence in laboratory (Long et al. 2008). Bekker et al. (2003) and Long et al. (2008) obtained a positive correlation between laboratory seed persistence and field persistence experiments. Dickie et al. (1990) determined temperature range that was detrimental to seed survival of eight diverse crop species.

Seed parameters such as seed size or mass and seed composition have also been considered to estimate seed longevity under laboratory conditions. Thompson et al. (1993) and Peco et al. (2003) illustrated a positive relationship between seed size and seed persistence. Hendry et al. (1994) attempted to estimate seed persistence based on concentration of ortho–hydroxyphenol, an antimicrobial compound. Phenol concentration in seeds was used by Holmes et al. (2004) to predict seed persistence in fire–prone communities such as fynbos.

Weed seed persistence from a management perspective is well studied and much research has been conducted for the development of efficient, site and species–specific weed management strategies. However, little is known about the seed persistence estimation of weed species under domestication. During the process of evolution, changes associated with different factors effecting seed persistence probably result in a change in seed persistence status in the soil seed bank.

3.0 MORPHOLOGICAL AND AGRONOMIC EVALUATION OF COW COCKLE (*Vaccaria hispanica* [P. Mill.] Rauschert) POPULATIONS

3.1 ABSTRACT

Domestication is the process by which a wild plant undergoes morphological, physiological or molecular changes and ultimately evolves into a crop. These evolutionary changes can be used to understand the transition from wild to cultivated plant. Cow cockle is a summer annual species introduced to North America from Europe. It has been considered for commercial production because of its seed constituents. The purpose of the study is to evaluate available cow cockle germplasm to determine the populations that are best adapted to the cultivation as well as the traits responsible to such adaptation. We compared 15 cultivated, weedy and wild cow cockle populations from different parts of the world for several agronomic and morphological traits at two sites (Saskatoon, SK and Edmonton, AB) from 2009 to 2011. Multivariate analyses were performed to group populations according to their agro–morphological similarity. Cluster analysis revealed three main groups among the populations based on the traits studied. Two linear discriminants were obtained by discriminant function analysis accounting for 96% of the variability among the populations. Analysis of variance showed significant ($P<0.001$) differences for most of the characters studied, however populations did not differ for emergence and disease resistance. Physiological maturity, seed size, plant height, flower size and seed yield were responsible for the divergence in cow cockle populations. The cluster including Pink Beauty, Turkey, PB–87, Scott and UMan–89 were characterized by taller plants (~58 cm), production of heavier seeds (~7.7 gm) and high seed yield (~2400 kg ha^{-1}). A high agronomic potential in these populations may be because of pre–adaptation towards domestication, which they might have acquired during the process of cultivation. These pre–adapted populations have potential to be utilized in future breeding programs of cow cockle. Although weedy populations showed some adaptation to cultivation, characters relating to its plant architecture, seed weight and yield may need further selection.

3.2 INTRODUCTION

Cow cockle (*Vaccaria hispanica* [P. Mill.] Rauschert) is an annual herb originating from Europe that belongs to the family Caryophyllaceae. It is widely distributed in Asia, Europe and North America (Shengmin et al. 2000; Meesapyodsuk et al. 2007). Cow cockle seeds resemble canola in size and shape, and cow cockle can be grown commercially with existing farm machinery (Goering et al. 1966). It is considered as a medicinal weed in China and India, and the seeds are used to treat diuresis, inflammations and to promote milk secretion (Kumar et al. 2011; Feng 2012). The seeds contain fine starch, cyclo–peptides and saponins which have the potential to make this species an important nutraceutical plant (Mazza et al. 1992; Sonnet et al. 2001; Efthimiadou et al. 2012). Balsevich et al. (2006) analyzed bisdemosidic saponins (anti–cancer) in three different populations of *Vaccaria hispanica*. Feng (2012) successfully demonstrated the angiogenic effect of cow cockle extract in chick models. In addition to the desirable seed constitution, cow cockle has been found to have very favorable agronomic characters, making it a potential candidate for domestication. Attempts towards commercialization of this species have been made in western Canada (Saponin Inc. 2003) including the development of a double haploid protocol (Microspore culture) for the rapid production of homozygous populations (Ferrie et al. 2005).

Cow cockle is one of the many weed species that were introduced to North America from Europe (Chestnut and Wilcox 1901). In Canada, it is currently considered an annual weed in grain fields, especially under fine textured soils (Balsevich 2008). Precisely how cow cockle was introduced into North America is unclear. Though considered a weed at present, it is believed to have been grown as an ornamental initially (Crandall 1893). Alternatively, there are reports suggesting its entrance into Canada (Manitoba) as a contaminant in flax seed shipment from Europe (Fletcher 1897)**.** During the period 1945–1965, cow cockle had a steep increase in abundance and distribution in the Prairie Provinces (Alex 1966) making it one of the worst weeds in some areas. Alex (1968), reported a considerable yield reduction in flax by cow cockle. It was also observed that cow cockle is resistant to the commonly used broad–leaf weed herbicides MCPA (2–methyl, 4–chlorophenoxyacetic acid) and 2, 4 –D (2, 4 – dicholorophenoxyacetic acid) (Molberg 1966). However, the frequency and relative abundance of this species has drastically decreased in recent weed surveys (Thomas et al. 2007), suggesting

a reduction in impact as a weed. Weed management practices employed in the Prairies such as herbicides and zero tillage were attributed for this reduction in weed populations (Harker et al. 2009).

Domestication involves the transformation of wild forms through a process of evolution resulting in cultivated plants (Fuller 2007). Cultivated descendants often show marked phenotypic differences when compared to their wild progenitors (Harlan 1992). These differences are due to different selection pressures; such as natural selection, genetic drift, mutation and as well as artificial selection (Price and King 1968) during the process of adaptation to man–made habitats (Schwanitz 1957; Harlan 1992). The discrepancies involved in the domestication transition may vary within species and between species as well. In most cases, these differences can be used to identify the wild forms from the domesticated (Schwanitz 1966; Gepts 2004) or the domestication trend of the species.

There are several stages in the evolution of domesticated crops (Evans 1976). During the initial domestication phase, adaptation to cultivation is the main criterion. However, when plants spread from their places of origin, adaptation to new habitats was emphasized and later increased yield potential to agronomic inputs was considered more important. Domestication of maize (*Zea mays* sub sp. *mays* L.) from its wild relative, teosinte (*Zea mays* ssp. *Parviglumis)* was associated with several changes in plant characters such as increased apical dominance, reduced lateral branching and evolution of synchronized maturation (Doebley 2004). Apart from the development of tough rachis, early cereals were selected for shorter plants to avoid lodging, large spikes (and grains) and disease resistance during the process of domestication. Lablab bean (*Lablab purpureus* L. *Sweet*), had several seed morpho–physiological changes such as changes in seed size, color, shape, seed coat thickness and dormancy during its well documented domestication process (Maass 2006). *Triticum dicoccoides* koern., has been identified as the progenitor for the cultivated emmer wheat (*Triticum dicoccum* Shuebl.) based on the variability in growth habit and spike characters. In addition to cytological similarities, numerous morphological characteristics were also utilized to identify the progenitors of several grain crops such as tetraploid emmer wheat (*T. turgidum* L.), barley (*Hordeum vulgare* L.), lentil (*Lens culinaris* Medikus), pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.) (Zohary and Hopf 2000).

Because of its economic potential, cow cockle has attracted some commercial and scientific interest. The domestication status of cow cockle is currently unknown to agronomists and breeders. Understanding the phylogenetic history of a species is a prerequisite for domestication and commercial production (Balogun et al. 2008). We hypothesize that among several populations of a species, some are more amenable to domestication when compared to other populations. Accordingly, the purpose of this study was to evaluate available cow cockle germplasm to determine the populations that are best adapted to cultivation as well as the traits responsible for such adaptation.

3.3 MATERIALS AND METHODS

3.3.1 Seed Source:

The study material comprised of 15 cow cockle collections: 9 populations were obtained from Prairie Provinces of Canada (Scott, UMan–00, 02, 04, 05, 88, 89, Scott Weedy and Saskatoon Weedy). Two lines each from the United Kingdom (Florist Rose and White Beauty) and Finland (Pink Beauty and PB–87), and single germplasm accession each from Turkey (Turkey) and Mongolia (Mongolia). A detailed description of these populations is reported elsewhere (Chapter 4).

3.3.2 General Procedures:

The field experiments were conducted at Kernen Crop Research Farm, Saskatoon, SK, Canada (59^{o} 09', 106^{o} 33') for three years (2009, 2010 and 2011) and at Edmonton, Alberta (53°25' N, 113° 33' W) in 2009. In 2009, 13 populations were used in both locations. At Saskatoon in 2010 and 2011 the "Scott Weedy" and "Saskatoon Weedy" populations were added for a total of 15 populations. All the populations were arranged in a randomized complete block design (RCBD) with four blocks. The populations were planted in 2 x 6 m plots on fallow or wheat stubble. The seeds were sown at a target plant density of 300 plants m^{-2}, where each seed was placed at a depth of 2 cm with a row space of 20 cm using a cone seeder with disc openers. The seeding rate for each population was adjusted based on the thousand seed weight (TSW), germination percentage and a 5% mortality rate. Nitrogen and phosphorus fertilizers were applied at the time of seeding according to the spring soil test recommendations for spring wheat. Prior to sowing, a pre–seeding application of glyphosate at 900 g a.e. ha^{-1} was applied to control

pre–emergence weeds. The plots received an in–crop application of clethodium (56 g a.i. ha^{-1}) at the 3–4 leaf stage (cow cockle) to control the grass weeds. In–crop broadleaf weed control was achieved by hand weeding at regular intervals until flowering. Plots were monitored for off–types throughout the cropping period and were removed upon detection. The rate of gene flow among the populations was insignificant, which was tested in a separate greenhouse experiment (data not shown). Based on disease incidence (Alternaria black spot), a fungicide spray of azoxystrobin (250 g a.i. ha^{-1}) at pre– or mid–flowering stage was applied to all the plots. All the other management conditions were similar over years and sites.

3.3.3 Data collection

Agronomic measurements and morphological characterizations were conducted as detailed in Table 3.1.

Table 3.1 List of agronomic and morphological characters analyzed.

Parameter	Growth Stage	Measurement
Emergence*	2 weeks after emergence	Number of seedlings in two 1 m row lengths obtained from front and back in each plot.
Plant Height*	Floral initiation stage	Based on 10 random plants from each plot.
Days to Flower	5% Flowering stage	Visual observation (on plot basis).
Number of Branches	Floral initiation stage	Based on 10 random plants from each plot.
Flowering Period	5 – 95% flowering period	Calculated based on the duration between days to 5% flowering to 95% flowering.
Flower Size	Full bloom stage	Measured based on 20 random flowers from each plot using vernier calipers.
Disease Resistance (Alternaria black spot)	6 leaf stage to full petal drop stage	10 random plants in each plot were examined and scored using a 1–5 disease rating scale (1–No symptom, 2–Moderate lesions on lower leaves, 3–Heavy lesions on lower leaves, 4–Severe lesions on lower leaves and 5–All leaves dead).

Table 3.1 List of agronomic and morphological characters analyzed.

Biomass	Prior to maturity	Based on the dry weights (60 C for 72 h) obtained from an area of 0.25 m^2 (quadrat) from both front and back in each plot.
Physiological Maturity*	60% seed color change	Measured based on 10 random plants from each plot.
Yield*	Harvest maturity stage	Seed yield was calculated based on a plot basis, border rows on either side of the plot were excluded from the harvest.
Thousand Seed Weight*	Post–harvest	Based on 3 replications of 1000 seeds.
Seed Shattering	Post–harvest	Area of 6 random quadrats (0.0625 m^2 each) with the entire crop residue, shattered seeds and some surface soil were removed using a vacuum; later sieved and cleaned to obtain the viable seed.

* Agronomic characters studied at Edmonton, 2009.

3.3.4 Statistical Analysis

A combined analysis of all the data from four site–years was performed using analysis of variance (ANOVA) with SAS mixed models (SAS Institute Inc., 2008). Means were separated using Fisher's protected Least Significant Difference (LSD) at $P < 0.05$. Patterns of morphological similarity or difference were analyzed by multivariate statistical methods including cluster analysis (CA) and discriminant function analysis (DFA). MASS and VEGAN packages of R software were used for multivariate analysis (R 2.10, http://www.R–project.org). Cluster analysis was performed using mean values to group the populations with similar agro–morphological characters and visual phylogenetic relations existing among them. CA was conducted by analyzing the standardized variables for each accession without any pre–classification using Gower's distance matrix. DFA was carried out on the observed groups to identify the characteristics that contribute most to the variability based on their absolute values in the linear discriminants. Since limited characters were measured in Edmonton 2009, replicated

data from the Saskatoon location was used to perform DFA. To avoid multi–collinearity, redundant variables were removed from the analysis. The basic difference between CA and DFA is group membership and number of groups. In the case of CA, both group membership and number of groups in the populations were unknown, whereas in DFA it is known.

3.4 RESULTS AND DISCUSSION

The cluster analysis based on 12 agronomic and morphological traits, assigned the 15 cow cockle populations into three main clusters (Figure 3.1). The first cluster consisted of all the putative weedy populations. All these populations were from Canada, with UMan–88, 00, 02, 04 and 05 collected in Manitoba while Scott Weedy and Saskatoon Weedy were from Saskatchewan. The populations in this cluster possessed similar physiological maturity, thousand seed weight, plant height and flower size (Figure 3.3, 3.4, 3.5 and 3.7).

Five populations (Pink Beauty, Turkey, PB–87, Scott and UMan–89) were included in the second cluster because of their similarities in maturity, thousand seed weight and seed yield. This cluster had mostly cultivated populations. Although UMan–89 was initially categorized as a weedy population; it was grouped with cultivated populations (Figure 3.1).

The remaining populations formed the final cluster, which included two cultivated ornamentals and Mongolia. Similar flower size, floral initiation and flowering period characterized for this grouping (Table 3.4; Figure 3.7). Although Mongolia possesses contrasting floral characters from other two populations, similarity in thousand seed weight probably resulted in its inclusion in this cluster (Figure 3.4).

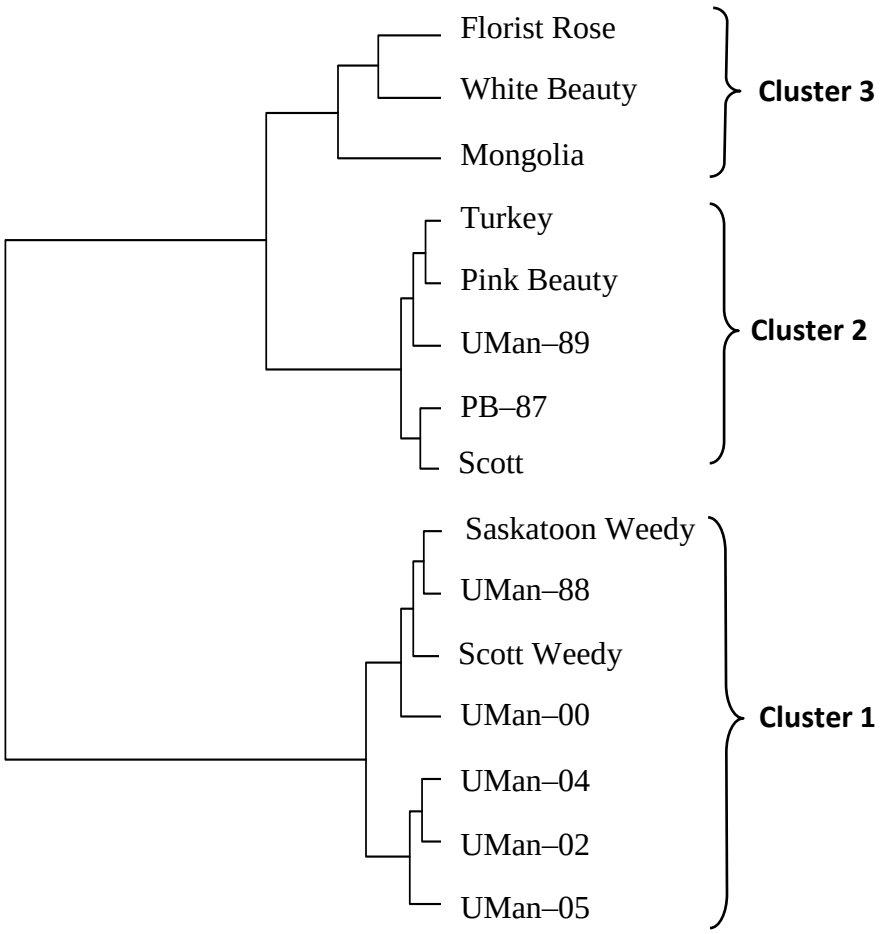


Figure 3.1 A dendogram of 15 cow cockle populations generated by hierarchical cluster analysis based on agronomic and morphological characteristics across four site–years.

Discriminant function analysis derived a set of linear discriminants based on agronomic and morphological variables. Linear discriminant is a linear combination of variables that best separates the groups or classes. Large eigenvalues for the first two linear discriminants suggest they are potentially good discriminators, and collectively they account for 96% of the variability in the populations (Table 3.2). Two of the predictor variables, days to flower and biomass showed high correlation (data not shown) with physiological maturity and plant height respectively and therefore were discarded from the analysis to avoid the multi–collinearity. The first linear discriminant accounted for 88% of the variability and subsequent discriminant explained 8% of the variation in the populations (Table 3.2). The coefficient of linear discriminants of DFA showed that physiological maturity, thousand seed weight, plant height, flower size and seed yield are the important characters that accounted for most of the variability among the populations (Table 3.2). For example, most of the Canadian populations were characterized by early maturity, smaller seeds and shorter plant architecture (Figure 3.3, 3.4 and 3.5), which they may have acquired during the process of adaptation to non–native environmental conditions upon introduction. In contrast, artificial selection pressure of cultivation may have resulted in the most populations of cluster 2, which are characterized by larger seed, robust plants and greater yields (Figure 3.4, 3.5 and 3.6). This may indicate the adaptive value of these traits in native or non–native environments in response to natural or artificial selection pressures.

Table 3.2 Standardized coefficients of the first two linear discriminants (LD) of the agronomic and morphological characters assessed in cow cockle.

Parameter	LD1	LD2
Emergence	–0.238	–0.843
Plant Height	–1.362	–0.606
Number of Branches	0.327	0.222
Flowering Period	–0.260	0.420
Flower Size	–1.160	3.370
Disease Resistance	–0.011	–0.270
Physiological Maturity	–4.030	–3.061
Yield	–1.473	0.217
Thousand Seed Weight	–2.777	0.482
Seed Shattering	0.015	0.026
Explained Proportion of Variance (%)	87.810	8.300
Cumulative Proportion of Variation (%)	87.810	96.110

The two–dimensional plot of discriminant function analysis shows distinct groups among the cow cockle lines (Figure 3.2). Physiological maturity and thousand seed weight were the most important variables in the first linear discriminant function (Table 3.2). However, flower size, plant height and yield also had some effect on the grouping. Based on the first linear discriminant axis (Figure 3.2), cluster 1 including all the weedy populations was separated clearly from the other groups (cultivated and ornamentals). This is due to the differences in both maturity and seed size. Although ornamental populations of cluster 3 differ from cultivated populations of cluster 2 in seed size, they were placed relatively closer to each other because of their similarity in maturity (Figure 3.3). In the second linear discriminant function, flower size and physiological maturity were important (Table 3.2). Ornamental populations (Cluster 3) with unique floral characters were grouped separately on the second linear discriminant axis (Figure 3.2). Late maturing and small flower diameter of Mongolia (cluster 3W) were responsible for its distant grouping from Florist Rose and White Beauty when compared to other groups (Figure 3.3 and 3.7). Mongolia was initially grouped with ornamental populations by cluster analysis; however DFA showed some evidence that Mongolia differs from ornamental populations (Figure 3.2) and hence will be discussed as a different group. Similar to cluster analysis, DFA also grouped UMan–89 and Scott with cultivated populations.

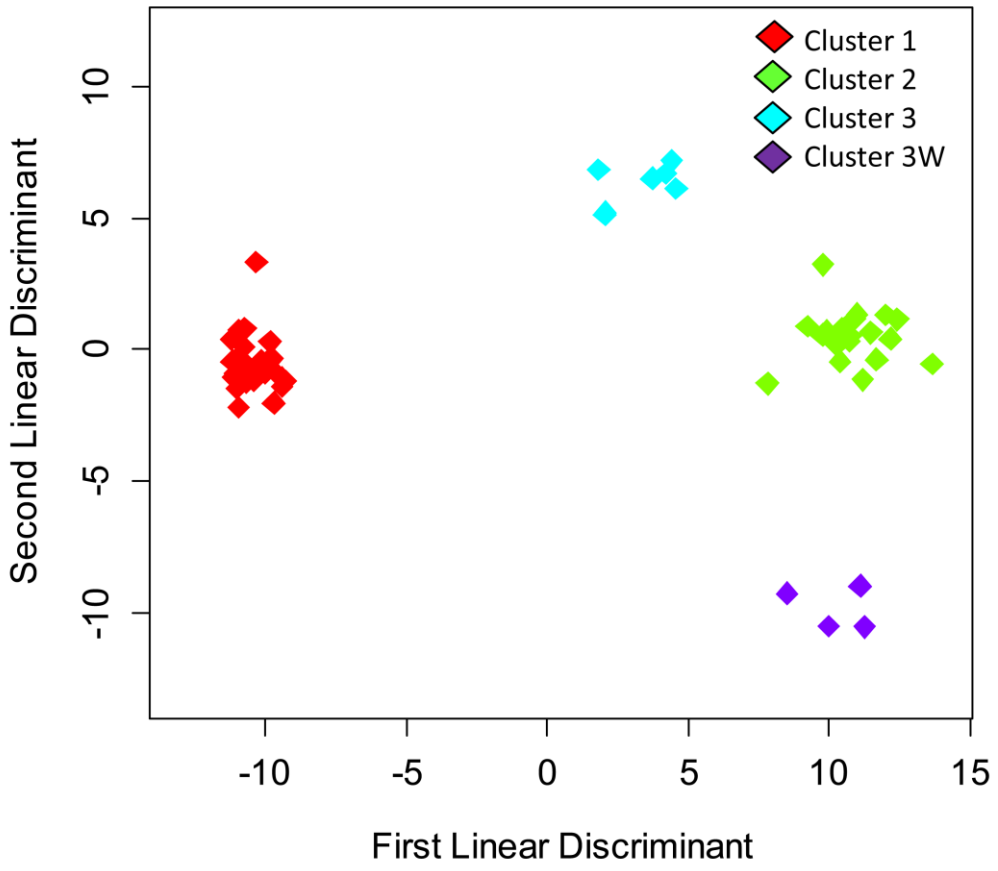


Figure 3.2 Classification of cow cockle (*Vaccaria hispanica* [P. Mill.] Rauschert) population clusters based on agronomic and morphological traits across three site–years at Saskatoon using discriminant function analysis of the first two discriminant dimensions.

Analysis of variance (ANOVA) revealed significant variability among the 15 populations studied for the variable measured (Table 3.3). A consistent variation for most of the characters studied suggests differences in the genetic background of the populations. Variation in the characters, plant height, days to flower, number of branches, flowering period, flower size, biomass, physiological maturity, yield, thousand seed weight and seed shattering was significant. However, the populations under investigation did not vary for emergence and disease resistance (Table 3.3). Furthermore, ANOVA was utilized to explore and quantify the differences in individual traits among the groups observed by the multivariate analysis.

Similar to the initial classification, all the weedy populations (except UMan–89) were grouped to form a single cluster (cluster 1) based on the agronomic and morphological characters studied (Figure 3.1). Interestingly, UMan–89 was clustered with cultivated lines. The populations in this group (UMan–88, 00, 02, 04, 05, Scott Weedy and Saskatoon Weedy) showed striking difference in growth habit from the other lines. The mean plant height of these populations was in the range of 35 to 42 cm, which is 30% less than most of the populations studied (Figure 3.5). Shorter plants with relatively high biomass (~700 g m^{-2}) may have evolved to support self–dispersal in these populations. Tumbling seed dispersal has been reported in cow cockle (Chestnut and Wilcox 1901), although not observed in the present study. At maturity, the plants break off at the base and the tops of the plants blown by the wind like tumbleweeds. Most weedy forms of cereals are adapted to disturbed environments but still maintained self–dispersal (Harlan et al. 1973). All the weedy lines from Manitoba (except UMan–89) and Saskatchewan showed early flowering (43 to 46 days) and maturity (83 to 85 days). The early maturity was found to be another potentially adaptive trait that may have developed in response to the short summers of Western Canada.

Table 3.3 Analysis of variance (ANOVA) for agronomic and morphological traits of cow cockle as affected by population, assessed at Saskatoon and Edmonton locations.

Source	EM	PH	DTF†	NOB†	FP†	FS†	DR†	BM†	PM	YD	TSW	SS†
	plants m^{-2}	cm	days	branches $plant^{-1}$	days	cm	1–5 scale	g m^{-2}	days	kg ha^{-1}	g	seeds m^{-2}
Siteyear	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Population	NS	***	***	***	***	***	NS	**	***	**	***	**
Siteyear x Population	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error	42.61	3.36	1.55	0.48	0.90	0.05	0.15	95.14	1.48	236.88	0.22	1.41

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

§ NS – denotes not significant.

† Data were analyzed from Saskatoon in 2009, 2010 and 2011.

‡ EM, Emergence; PH, plant height; DTF, days to flower; NOB, number of branches; FP, flowering period; FS, flower size; DR, disease resistance; BM, biomass; PM, physiological maturity; YL, yield; TSW, thousand seed weight; SS, seed shattering.

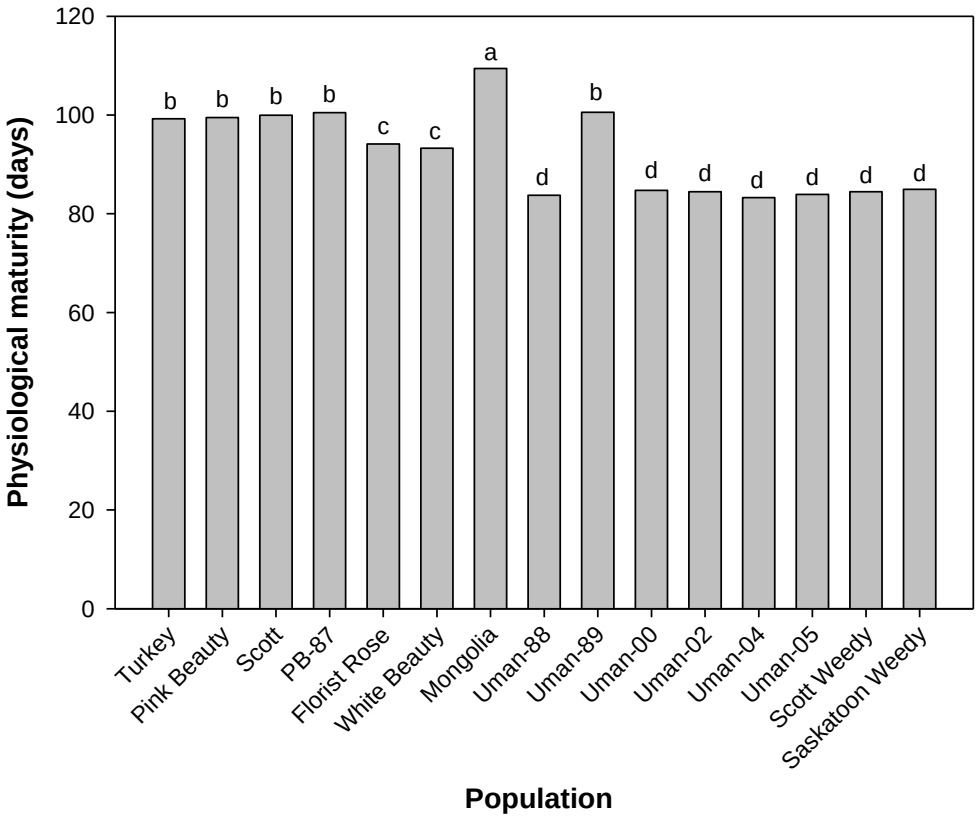


Figure 3.3 Physiological maturity of cow cockle populations assessed at Saskatoon (2009, 2010 and 2011) and Edmonton (2009) locations. Comparisons were made among populations; means followed by the same letter are not significantly different at $P < 0.05$.

All the populations of cluster 1 produced smaller seeds; the thousand seed weight (TSW) was significantly different from other populations, which ranged from 3.2 to 3.7 g (Figure 3.4). The reduced seed size in these populations may have developed as an adaptation in the new environment upon introduction. The seed size of early introduced cow cockle in North America was documented to be twice the size of commercial mustard (Chestnut and Wilcox 1901), however the seed size of these populations is relatively small, which may be comparable to the size of wild mustard seed. Production of smaller seeds in many weedy forms is function of survival, as small and round seeds facilitate easy burial (Thompson et al. 1994) and avoids predation (Hulme 1998). In addition, intermediate yields (~1300 kg ha^{-1}) of these populations may not limit their survival, as their small seed size would supply enough propagules for the next generation (Figure 3.6). According to De Wet (1975) weed races can develop either from domesticates (feral) or wild colonizers through selection. When wild colonizers are distributed to a non–native range, weedy races can be formed through the process of natural selection in order to adapt the new conditions (habitat disturbances). These weeds flourish in the new habitats based on their invasive ability, which is true in the present populations from Canada.

Weeds can become domesticated either through convergent and divergent adaptation (Kupzow 1980). Convergent groups of weeds are easy to domesticate as they evolve along with crop plants by adapting to the conditions of cultivation. For example, sweet clover (*Melilotus albus* Desr.), an introduced and naturalized weed species in the U.S. has been domesticated as a forage and cover crop. In contrast, divergent weed populations are difficult to domesticate as they tend to adapt to escape the methods of cultivation. For example, Canada thistle (*Cirsium arvense* [L.] Scop.) has been introduced from Eurasia to Canada, where it is classified as a primary noxious weed seed. In the present weedy populations, uniformity in emergence, flowering and maturity suggests a crop–like behavior; however characters such as early flowering, maturity and production of smaller seeds may have evolved to escape the weed control methods, suggesting a divergent adaptation. Synchronous tillering and ripening has been one of the key characters of domestication syndrome, especially in cereals (Pickersgill 2007). Unlike cultivated cereals, wild populations are often characterized by production of several unbranched culms that flower at different times (De Wet 1975). Sahli et al. (2008) suggested that evolution of early flowering time as one of the key adaptations responsible for the success of wild radish (*Raphanus raphanistrum* L.) as a major agricultural weed. Although these

populations showed some characters that adapt to cultivation, a majority of the traits suggest a weedy habit. Synchronized growth behavior of weedy populations suggests an adaptation to cultivation; however this may be true because the process by which weeds evolved in agro–ecosystems is similar to that of crops (Harlan and De Wet 1965). However, it has also been argued that weediness is part of pre–adaptation to domestication (Hawkes 1983).

The present phenotypic evidence allowed us to speculate that the weedy populations (cluster 1) are the variants of an early introduced species, which might have adapted to naturalize in its non–native range. In addition, none of the other populations (cluster 2, 3 and 3W) in the study showed similar characteristics. Adaptation of introduced populations to a wide range of new habitats was reported in several species. For example, Velvetleaf (*Abutilon theophrasti* Medik.) is an introduced weed from China to North America. In a study to understand the relationship between life–history characters and allozyme variation of 39 populations of velvetleaf, Warwick and Black (1986) reported significant differences in 33 out of 51 characters that represent the first stages of differentiation in response to local environment. Similarly, in Europe, the introduced populations of *Solidago* species showed significant variation in morphological and life–history characters (Weber and Schmid 1998).

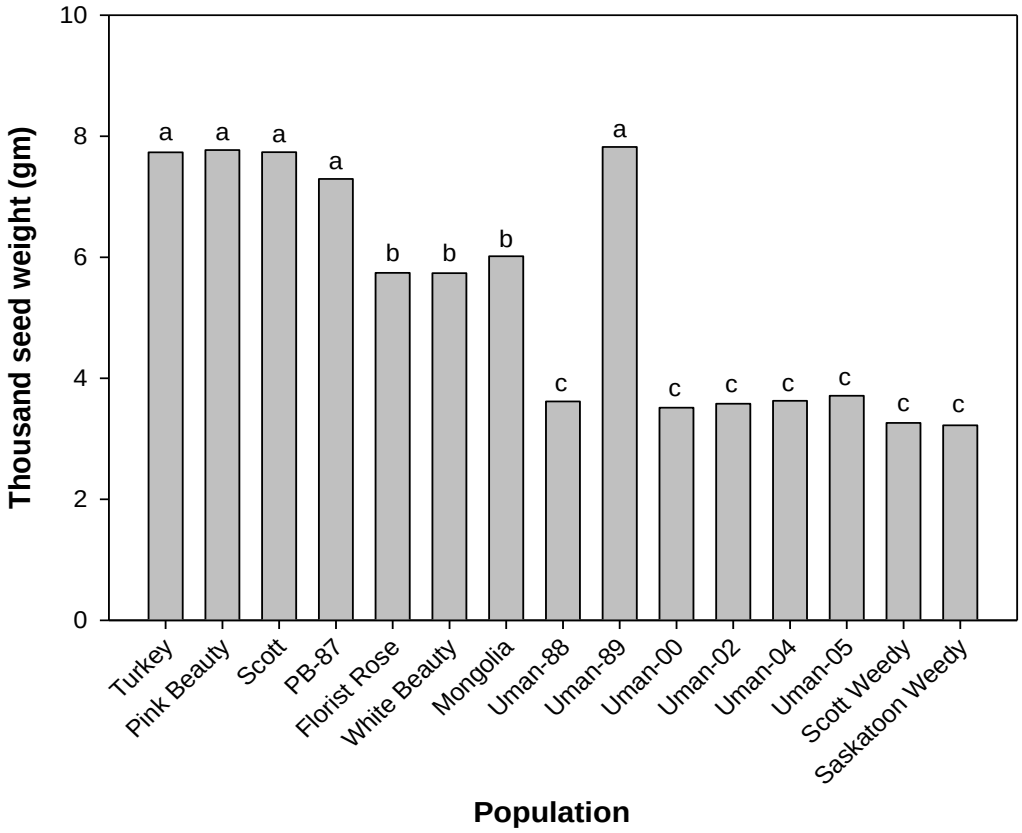


Figure 3.4 Thousand seed weight of cow cockle populations assessed at Saskatoon (2009, 2010 and 2011) and Edmonton (2009). Comparisons were made among populations; means followed by the same letter are not significantly different at P < 0.05.

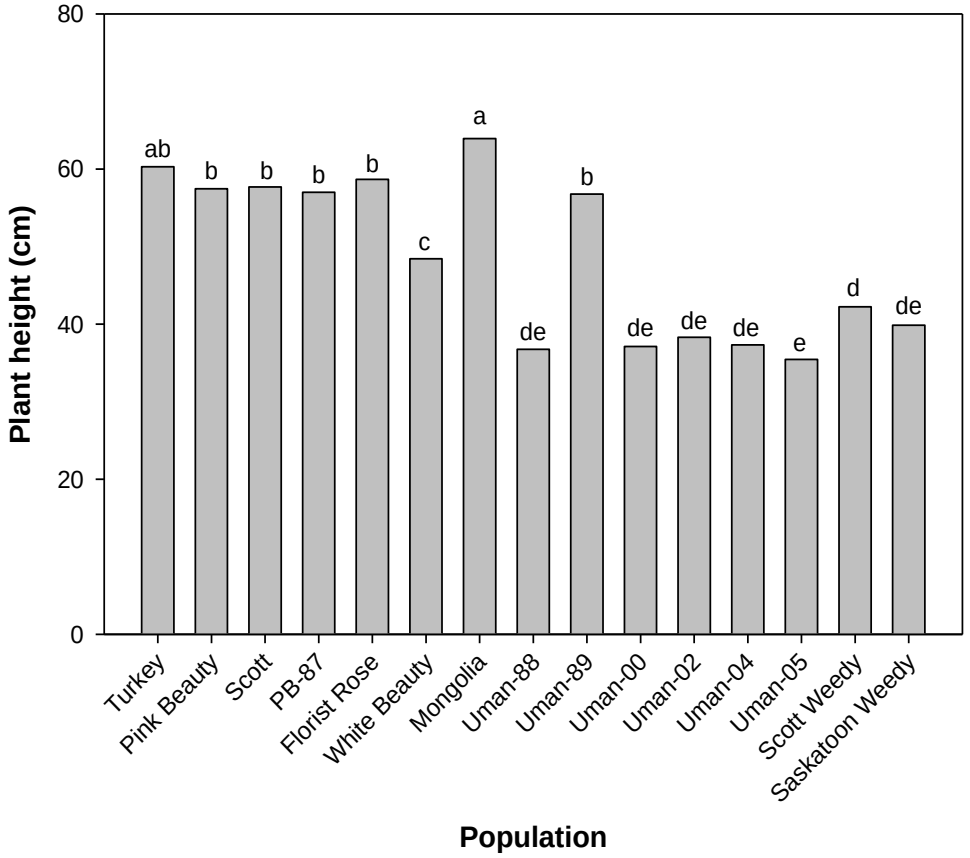


Figure 3.5 Plant height of cow cockle populations assessed at Saskatoon (2009, 2010 and 2011) and Edmonton (2009). Comparisons were made among populations; means followed by the same letter are not significantly different at P < 0.05.

Table 3.4 Means values of agronomic and morphological characteristics of cow cockle (*Vaccaria hispanica* [P. Mill.] Rauschert) populations assessed at Saskatoon and Edmonton.

Population	EM	DTF	NOB	FP	DR	BM	SS
	plants m^{-2}	days	branches $plant^{-1}$	days	1–5 scale	g m^{-2}	seeds m^{-2}
Pink Beauty	171	54	9	9	3.0	1033.8	8
Turkey	171	54	8	9	3.1	977.9	7
Mongolia	158	64	11	7	3.0	878.7	6
Scott	157	51	9	8	2.9	977.7	8
UMan–88	156	43	11	8	2.9	682.0	10
White Beauty	119	49	12	11	3.0	586.7	4
PB–87	150	50	10	8	2.6	905.8	10
Florist Rose	168	52	13	12	2.7	773.9	6
UMan–89	148	51	10	9	2.7	1075.5	8
UMan–00	129	43	9	8	3.0	612.9	11
UMan–02	146	45	10	8	3.1	720.6	6
UMan–04	183	45	9	8	2.7	693.8	7
UMan–05	113	45	8	8	2.8	687.3	6
Scott Weedy	145	45	12	6	3.0	621.3	10
Saskatoon Weedy	150	46	11	9	3.0	623.1	10

‡ EM, Emergence; DTF, Days to flower; NOB, Number of branches; FP, Flowering period; DR, Disease resistance; BM, Biomass; SS, Seed shattering.

The group of cow cockle populations (cluster 2) including Pink Beauty, Turkey, PB–87, Scott and Uman–89 are characterized by taller plants, high biomass (~990 g m^{-2}), higher grain yields and heavier seeds (Table 3.4; Figure 3.1). The mean plant height of these populations was only 10% less than the tallest population, Mongolia (Figure 3.5). The average flower diameter of these populations is 1.3 cm, which is almost twice the size of weedy and wild populations (Figure 3.7). These populations were medium in physiological maturity, as they took two weeks longer than the weedy lines and a week less than Mongolia to mature (Figure 3.3). Interestingly the seed yields of these populations are comparable with most modern domesticated crops (Figure 3.6). The mean yield of these populations was greater by 72% when compared to the populations studied in a previous study (Goering et al. 1966). UMan–89 is the only putative weedy population that had greater yield and larger seeds when compared with other weedy populations in the study.

Cultivation provides conditions that favor increased seed yield by reducing the competition from other species and increasing seed size because of intense intra–population competition (Harlan et al. 1973). To the best of our knowledge, there was no conscious effort to select for seed yield and thousand seed weight in this species other than in the cultivated population, Scott which may have been selected unintentionally for larger seed size. An increase in seed size under cultivated field conditions has been reported in many cereals (Harlan et al. 1973; Purugganan and Fuller 2011) and legumes. In addition to seed weight, Scott had other plant characteristics similar to the cultivated populations, Pink Beauty, Turkey and PB–87, that suggest that all these populations may had undergone similar selection pressures either intentional or unintentional. The other population that resembles the cultivated lines is UMan–89, which was originally reported as a weedy population. However, at this moment, we believe that the selection pressures that resulted UMan–89 may be different from other weedy populations in the study.

The populations in cluster 2 (Figure 3.1) appear to have certain pre–adaptation towards domestication. It is not known for how long these populations were under cultivation; here we propose that pre–domestication cultivation may be responsible for the morphological adaptations in these populations. Several studies demonstrated that some species are easier to domesticate in relation to characteristics that pre–adapt them to domestication (Rindos, 1984; Diamond, 2002).

Furthermore, archeological evidence reveals that humans cultivated non–domesticated plants for millennia before they became fully domesticated (Purugganan and Fuller 2011). However, studies on intra–population variation in pre–adaptation are limited. The pre–adapted variability among these populations is expected to be useful for further breeding programs.

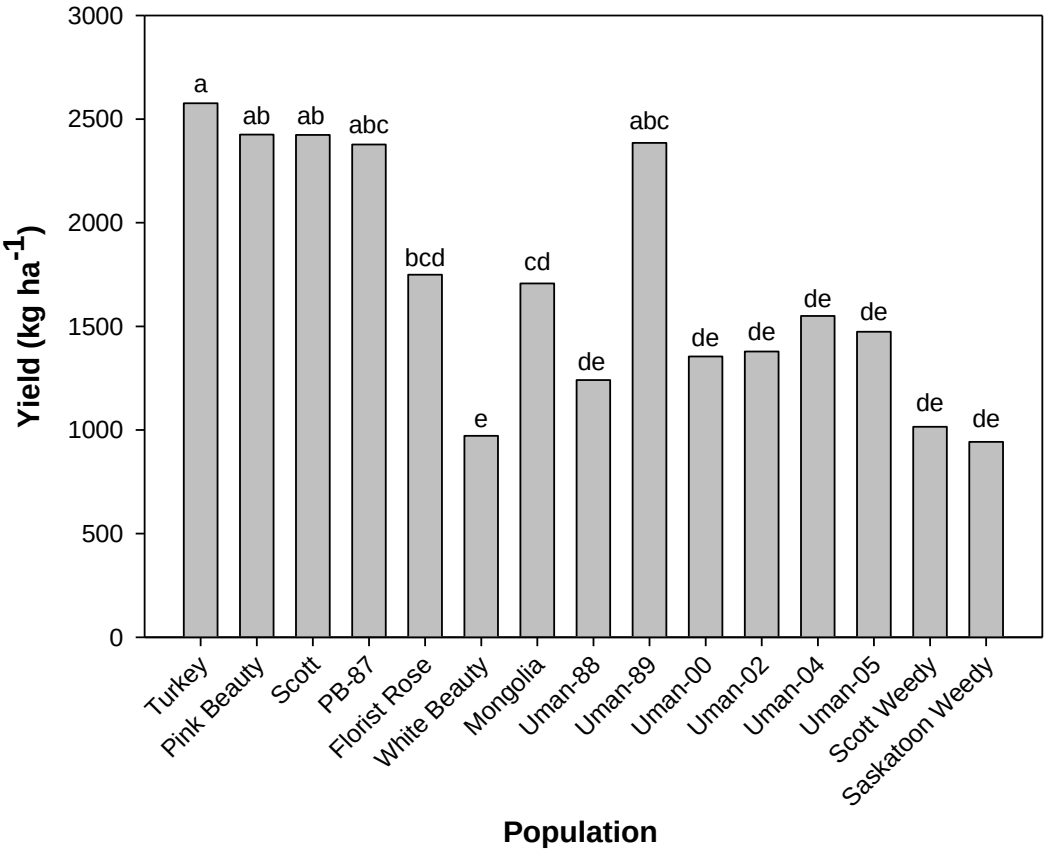


Figure 3.6 The yield of cow cockle populations assessed at Saskatoon (2009, 2010 and 2011) and Edmonton (2009). Comparisons were made among populations; means followed by the same letter are not significantly different at P < 0.05.

The two ornamentals, White Beauty and Florist Rose (cluster 3) were clustered separately from other cultivated populations because of their distinct floral characters (Figure 3.1 and 3.7; Table 3.4). Selection of aesthetically pleasing variants is a prerequisite for ornamentals (Hawkes 1983). The floral initiation of ornamentals was similar to other cultivated lines, but flowers remain open (flowering period) for a longer period in both White Beauty (11 days) and Florist Rose (12 days) (Table 3.4). Quality, longevity and inflorescence architecture are considered important in domestication of ornamentals (Waiganjo et al. 2008). Introduction of *Iris* species (subspecies *Oncocyclus*), native of southwest Asia, as a major cut flower has been only marginally successful due to its short vase life (Weiss 2002). Flower size of White Beauty and Florist Rose was 1.7 and 2 cm diameter respectively, which is on an average 30% larger than the cultivated and 300% larger than weedy or wild populations (Figure 3.5). These marked floral characters may have been the result of the artificial selection, since these ornamentals were reported to be domesticated populations. Brits (1983) proposed three basic phases of the development process of an ornamental plant, starting from wild plant to commercially cultivated plant. First, the direct use of the wild populations; second, through basic domestication and third is through clonal selection. In the case of White Beauty and Florist Rose, we believe that these populations have been subjected to basic domestication which involves introduction of wild plants to cultivation; and subsequent selection for floral characters such as size, flowering period. Characteristics such as physiological maturity and plant height of ornamentals were similar to other cultivated populations (Figure 3.3 and 3.5). Intermediate seed size and poor seed yields of ornamentals suggest that these traits may be of less importance during their selection (Figure 3.4 and 3.6). This differentiation of ornamentals from others could be related to human alteration of populations selecting in favor of better phenotypes.

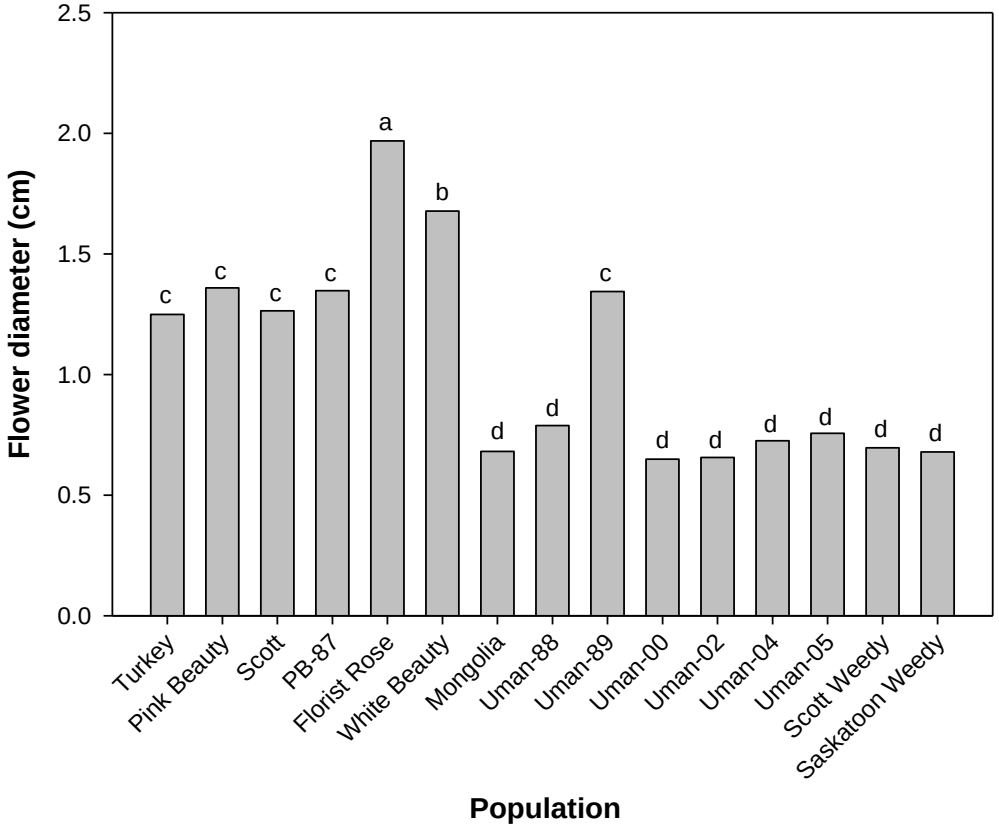


Figure 3.7 Flower diameter of cow cockle populations assessed at Saskatoon (2009, 2010 and 2011). Comparisons were made among populations; means followed by the same letter are not significantly different at P < 0.05.

The wild population Mongolia representing cluster 3W (Figure 3.2) is characterized by tall plants, late maturing and small flowers. Although it is grouped with the ornamentals, deviations in floral characters placed this accession separately in the dendrogram (Figure 3.1). Mongolia showed a longer vegetative period and later maturity compared with the other populations (Table 3.4; Figure 3.3). This late maturing characteristic may have been selected in relation to its native habitat. The Mongolia (PI 597629) line had originally been collected from a desert–steppe ecological zone generally dominated by perennial grasses or forbs (Sasaki et al., 2008). The seed size is similar to ornamentals, but flower size is in the range of weedy populations (Figure 3.4 and 3.7). However, most cultivated populations produced larger flowers. The limited knowledge on phenological history of Mongolia leads us to speculate that the characters observed in the study may have developed to adapt the conditions of native habitat.

The cow cockle populations were classified into four groups based on the phenotypic variability. Physiological maturity, seed size, plant height, flower size and seed yield differed the most in the populations and contributed to the differential clustering. The four clusters identified populations that were broadly grouped into weedy, wild, ornamental and cultivated. All the populations are adapted to cultivation, but the cluster including Pink Beauty, Turkey, PB–87, Scott and UMan–89 showed higher seed yield, larger seeds, and greater biomass than other clusters. This suggests that these populations are pre–adapted to domestication or partially domesticated. Future work of breeding for agronomically adapted cow cockle populations should utilize these populations.

PROLOGUE (CHAPTER 4)

Seed dormancy has been an important characteristic, which ensures distinct differentiation between domesticated and other categories (wild or weed) in the evolutionary trend. Rapid uniform germination is a characteristic of crops. In crops, a lack of dormancy is advantageous as it produces dependable germination and a predictable plant stand in the field. In contrast the presence of seed dormancy is an adaptive trait in wild or weed plants, which helps in timing the germination under most ideal conditions. Therefore a key step in the process of crop domestication is the loss of seed dormancy and evolution of uniform seed germination. As a part of understanding the domestication status of cow cockle populations, a study was initiated to evaluate seed dormancy and germination patterns in cow cockle populations (Chapter 4). Freshly matured seeds of all the populations showed high levels of primary conditional dormancy except "Mongolia". Optimum germination temperature for cow cockle was observed to be 10 C, as most populations germinated without any requirement of dormancy–breaking factors such as fluctuating temperatures or light. Mean temperature is more important than temperature regime and light for cow cockle germination. Although high seed dormancy is undesirable in domesticates, a complete elimination of dormancy may result in problems, such as pre–harvest sprouting especially under cold and humid environments (Gubler et al. 2005). Therefore, if cow cockle were grown as a crop, the conditional dormancy may not be considered a barrier to domestication and can be viewed as a physiological mechanism to avoid germination at harvest. However, as a weed, conditional dormancy may promote the formation of persistent soil seed banks.

4.0 VARIATION OF SEED DORMANCY AND GERMINATION ECOLOGY OF COW COCKLE (*Vaccaria hispanica* [P. Mill.] Rauschert) POPULATIONS

4.1 ABSTRACT

Cow cockle, an introduced summer annual weed of the Northern Great Plains, is being considered for domestication because of its high quality starch, cyclo–peptides, and saponins. Loss of seed dormancy is one of the key desirable traits for domestication. To determine the potential for domestication of this species, an understanding of the seed dormancy and germination patterns is required. The objectives of this study were i) germplasm evaluation to determine seed dormancy in cow cockle populations ii) to determine how temperature and light affect seed dormancy and germination. We evaluated 15 populations of cow cockle for primary dormancy by exposing them to 5 temperatures (5, 7.5, 10, 15, and 20 C) under two temperature regimes (constant and alternating) in both dark and light conditions. Freshly matured seeds of all the populations showed high levels of primary dormancy except "Mongolia". Lower levels of dormancy at medium temperatures (10 and 15 C) and greater dormancy at low and high temperatures suggest conditional dormancy, a state at which seeds germinate over a narrower range of conditions compared to non–dormant seeds. The effect of temperature regime, light and their interaction was significant only at sub–optimal (5 and 7.5 C) and supra–optimal (20 C) temperatures. Under these conditions, alternating temperatures were more effective in breaking the conditional dormancy, followed by light. The variation in optimum temperature, light, and their interactions among the cow cockle populations may be due to the plants evolving to adapt to their local environments. From a domestication perspective, the conditional dormancy in cow cockle can be observed as an evolutionary mechanism that prevents untimely germination following maturity and may not be a major obstacle for its domestication.

4.2 INTRODUCTION

Domestication is the evolutionary adaptation of an organism under human influence (Fuller and Allaby 2009). The shift from a nomadic hunter–gatherer system to a settled agriculture occurred with the aid of domesticated plants (Meyer et al. 2012). Tracking the origin of domesticates through their adaptations and underlying mechanisms is imperative to understanding the domestication process (Diamond 2002). Crops can be categorized as primary and secondary, based on their domestication pattern (Acquaah 2012). Primary crops are derived from wild progenitors, whereas secondary crops are evolved from weeds that grew within primary crops. A considerable number of Old World crops have entered the crop assemblage through domestication of their initial weedy forms (Vavilov 1926). Identifying useful products of a weed species and then deliberately planting these species helps transform the weed into a crop.

Cow cockle, also known as cowherb or china cockle, was first introduced to North America as an ornamental plant from Eurasia (Frankton and Mulligan 1987). A self–pollinated member of the Caryophyllaceae family, it was once commonly found in grain fields and abandoned areas (Chater 1964) and is now a minor summer annual weed found in the fine textured soils of the Northern Great Plains. Cow cockle may become a valuable secondary crop because it has a large seed, good plant vigor and produces acceptable yields (Goering et al. 1966). However, the main reason it is being considered for domestication is because of seeds and its phytochemical products. Cow cockle seed is a source of high–quality starch, cyclopeptides, and saponins: its ultra–fine starch granules (composing 60–65% of the seed) can be used in the cosmetic industry (Mazza et al. 1992); cyclic peptides (12–14%) have a wide range of anti–microbial properties (Mazza et al. 1992; Biliaderis et al. 1993; Sonnet et al. 2001); and saponins (2–4%) are known to have anti–cancer properties (Balsevich et al. 2006).

Seed dormancy is one among the important characters transformed during the process of domestication (Harlan 1992; Gepts 2002). Domestication of most Old World crops, especially pulses, was accomplished by reducing their wild modes of germination regulation such as seed dormancy (Ladizinsky 1987). Primary dormancy is genotypic in nature and possessed by seeds when they are dispersed from the mother plant. The fundamental role of primary dormancy is to time germination so that the probability of seedling survival is maximized. Primary dormancy is

an important adaptive trait in many undomesticated species (Baskin and Baskin 1988) because it helps them survive in variable environments by enabling seed to germinate over time. Primary dormant seeds can be completely dormant or conditionally dormant. Conditional dormancy is known as a transitional stage between dormancy and non–dormancy; a stage at which seeds germinate in a narrower range of conditions than non–dormant seeds (Baskin and Baskin 1998).

Cow cockle is a typical summer annual weed species and germination of fresh cow cockle seed is often low to negligible but increases with GA_3 treatment (Hsiao 1979). This suggests that cow cockle is likely to have non–deep physiological dormancy. Of the many environmental factors that regulate seed dormancy in annual weed species, temperature and light are the most important (Batla and Benech–Arnold 2010). In preliminary studies, freshly harvested cow cockle seeds have been observed to germinate better in the field than in the laboratory, likely due to fluctuations in soil temperature (data not shown). Many summer annuals require fluctuating temperatures (Benech–Arnold et al. 2000; Thomas et al. 2006) and light to break seed dormancy (Benech–Arnold et al. 2000). Hsiao (1979) reported a inhibitory effect of light on cow cockle seed germination. According to his study, when cow cockle seeds imbibed in water for 1 hr and then germinated, a higher germination percentage was observed in dark (77%) compared to light conditions (46%). However no such effect was observed on the germination of dry seeds.

Dormancy is undesirable in crop seeds because it reduces germination and subsequently causes uneven plant emergence (Evans 1996). Therefore a critical step in the process of crop domestication is the loss of seed dormancy and evolution of uniform seed germination. Because cow cockle is being considered for domestication, understanding its germination requirements and dormancy strategies is important. Other than a few preliminary studies, no research to date has examined the ecology of cow cockle seed dormancy and germination. In this study, 15 cow cockle populations were examined to measure primary seed dormancy levels in cow cockle and to determine how temperature and light affect seed dormancy and germination.

4.3 MATERIALS AND METHODS

4.3.1 Seed Source

For this study, 15 cow cockle populations were used.

"Mongolia" (*Vaccaria hispanica* [P. Mill.] Rauschert) was named after its place of origin, Mongolia. The seeds of this population were obtained from the USDA–ARS, North Central Regional Plant Introduction Station., accession number is PI 597629 (John Balsevich, Senior Research Officer, NRC–PBI, personal communication). Mongolia was considered wild material based on the USDA–ARS, Germplasm Resources Information Network database.

"Pink Beauty" and "Turkey" (*Vaccaria hispanica* [P. Mill.] Rauschert) are cultivated populations from Finland and Turkey respectively. The seeds of these lines were obtained from USDA–ARS, North Central Regional Plant Introduction Station, accession numbers are PI 578121 and PI 304488, respectively (John Balsevich, Senior Research Officer, NRC–PBI, personal communication). "PB–87" is derived by single seed descent from Pink Beauty in Saskatoon, 2002. Pink Beauty and Turkey were considered cultivated based on the USDA–ARS, Germplasm Resources Information Network database. The only putative semi–cultivated population in the study material is "Scott". The "Scott" line of cow cockle was an unconscious selection from weed control studies at Scott Research Station, Saskatchewan, Canada; which was originally collected near Regina, Saskatchewan during the 1970s. The seed multiplication for several generations (6 –10) resulted in a relatively homogenous landrace named after the Research Station as "Scott" (Eric Johnson, Weed Biologist, Agriculture and Agri–Food Canada, personal communication).

"White Beauty" (*Vaccaria hispanica* [P. Mill.] Rauschert) is a domesticated ornamental population from the United Kingdom, obtained from CN seeds, Denmark House, Pymoor, Ely, Cambridgeshire, CB6 2EG, United Kingdom (John Balsevich, Senior Research Officer, NRC–PBI, personal communication). "Florist Rose" (*Vaccaria hispanica* [P. Mill.] Rauschert) is also a domesticated ornamental population from the United Kingdom. The seed was obtained from B and T World Seeds, Paguignan, 34210 Aigues–Vives, France (John Balsevich, Senior Research

Officer, NRC–PBI, personal communication). White Beauty and Florist Rose were categorized domesticated as they can be obtained commercially.

All the Manitoba lines ("UMan–88", "UMan–89", "UMan–00", "UMan–02", "UMan–04", "UMan–05") were obtained from University of Manitoba, Canada. They were reported to be different weedy populations collected in Manitoba and were probably grown only once previous before this study (Christian Willenborg, Assistant professor, University of Saskatchewan, personal communication). The "Scott Weedy" population was from the Scott Research Station, Saskatchewan, Canada, obtained from weed seed cleaned from lentil seed following harvest in 2007. The "Saskatoon Weedy" population was similarly obtained in 2009 from an organic wheat grower of Saskatchewan.

4.3.2 Seed Multiplication

To minimize effects of climatic differences during seed development, maturation and collection, seed multiplication of all the populations was conducted in a common nursery with standard cropping practices. The rate of gene flow among the populations was insignificant, which was tested in a separate greenhouse experiment (data not shown). The common nursery was located at the Kernen Crop Research Farm, Saskatoon, SK, Canada (lat 59^{o}09' N, long 106^{o}33' W). The plots were harvested with a small plot harvester, with two rows left on either side. Seeds were cleaned with Carter Day dockage tester (Cea–Simon Day Ltd.) and seed blower (Fasco Industries, D127). To reduce moisture for safe storage, the cleaned seeds were air dried at room temperature (20–25 C) for 10 days. Finally, 100 g of seeds were randomly sampled for each population and stored at –80 C (Sanyo VIP Series, MDF–U71V) until the start of the experiments.

4.3.3 Temperature and Light

This study included a factorial distribution of temperature and light treatments. Temperature included 2 sub–factors: mean temperature and temperature regime (i.e., constant temperature and alternating temperatures). The light treatments included continuous dark (i.e., 24 h darkness) and light (i.e., 12 h/12 h dark/light). The seeds were subjected to 5 mean

temperatures (5, 7.5, 10, 15, and 20 C) under constant and corresponding alternating temperature regimes (0/10, 2.5/12.5, 5/15, 10/20, and 15/25 C) in both dark and light environments.

The study was conducted in the phytotron at the College of Agriculture and Bioresources, University of Saskatchewan, SK, Canada. A series of versatile environmental chambers (Sanyo, MLR–350H, Sanyo Scientific, USA) were used. Ten temperatures (5 constant and 5 fluctuating temperatures) were randomly assigned to each incubator, which were programmed to maintain defined temperature and cyclic light treatments. For light treatments, each chamber had twelve 40–W Sanyo tube lights (70–80 µmol $m^{-2}s^{-1}$). For dark treatments, the germination trays were covered with 2 layers of black plastic bags (Glad® Easy–Tie, 31 x 42 cm). The middle 3 shelves of each incubator were used to avoid any temperature fluctuations among the shelves within the incubator.

4.3.4 Germination Test

The dormancy assay began in February 2011. In each incubator, 5 replicates of 50 seeds per population were distributed in completely randomized experimental design at weekly intervals. The seeds were distributed in 9–cm petri dishes (BD Falcon ™, 100 x 15 mm standard style) lined with double–layered filter paper (Grade 22, Whatman Inc., USA) that had been moistened with 5 ml of distilled water. To prevent fungal growth during the experiment, 1 ml of 0.05% (v/v) benomyl solution was added to each petri dish. Supplementary experiments showed no adverse effect of 0.05% (v/v) benomyl on cow cockle germination. To reduce evaporation, clear plastic bags (Ziploc®, XL, 60 x 51 cm) were used to seal the germination trays. Approximately 2 mL of deionized water was added to petri dishes periodically to ensure the filter paper remained saturated throughout the testing period.

Germination counts were recorded for every 48 h during the 12 h light cycle of the light treatment (i.e., 12 h dark / 12 h light). A green safelight was used during the counting of the dark–germinated seeds. Seeds with ≥ 2 mm radical protrusion were considered germinated; the test continued until there was no further germination for 2 weeks. Temperatures of the germination chambers were recorded at hourly intervals using a Campbell 21X data logger. Germinated and rotten seeds were removed after each counting. A crush test (Sawma and Mohler 2002) was performed to determine the viability of the non–germinated seeds and to exclude non–

viable seeds from dormant seeds. Final germination was calculated based on the total viable seeds adjusted to 100%.

4.3.5 Statistical Analysis

The experiment was run twice with 5 replicates each per run. Data were pooled over the experimental runs, as there was no significant effect of repetition. Data were analyzed with a 4–way factorial ANOVA model, with population, mean temperature, temperature regime, and light as main factors. When the ANOVA suggested a significant main effect or interaction, Fisher's protected LSD tests were used for mean separation. All ANOVA and mean separation calculations were performed using PROC GLM in SAS (SAS version 9.2, SAS Institute Inc., Cary, NC, USA.).

4.4 RESULTS AND DISCUSSION

4.4.1 Primary Dormancy

In the present study, freshly harvested seeds of most cow cockle populations were characterized by high (> 85%) germination percentages only under certain given conditions (Figure 4.1). The germination of freshly–matured seeds at some temperatures without any dormancy–breaking factors such as alternating temperature or light, suggests that cow cockle is primary conditionally dormant. Seeds that germinate only under a narrow range of conditions are considered conditionally dormant (Baskin and Baskin 2004). For example, Copete et al. (2009) reported a conditional dormancy/non–dormancy cycle in two Iberian annuals, germinating at low and medium temperatures but not at high temperatures. Primary dormancy acts as a timer to ensure spring germination in summer annuals (Baskin and Baskin 1988) after cold stratification in the winter.

4.4.2 Population, Temperature and Light Interaction

Across the populations, temperature and light were found effective in relieving seed dormancy, however there was a signficant interaction between these two factors (Table 4.1). The effect of temperature and light interaction on seed dormancy was highly variable among the populations (Figure 4.1). However the effect of these interactions was minimal at 10 and 20 C, as

there were the least differences in dormancy between treatments and populations at these temperatures. For all the populations, dark treatment combined with a constant temperature regime resulted in the least seed dormancy, or both the light and the dark treatment at constant temperatures gave the lowest seed dormancy, with no difference between light and dark (Figure 4.1). Several studies have shown the importance of temperature and light in weed seed germination and seasonal dormancy cycles (Chauhan and Johnson 2010; Jha et al. 2010). Based on their responses to temperature and light treatments, populations were grouped and explained as follows.

***Mongolia*.** Mongolia is a non–dormant population of cow cockle. At mean temperatures of 5, 7.5, 10, and 15 C, its rate of dormancy ranged from 0 to 4% (Figure 4.1). No significant differences in seed dormancy were observed at these temperatures under different temperature regimes and light combinations.

***Turkey, Pink Beauty, Scott, PB–87, and UMan–89*.** These populations produced similar seed dormancy patterns under all the temperature regimes and light treatments (Figure 4.1). Regardless of light treatments, low dormancy levels at constant temperatures of 10 and 15 C suggests conditional dormancy of these populations. At the remaining temperatures (5, 7.5 and 20 C), these populations either require alternating temperatures or light or both to release the dormancy. Seeds that germinate only under a narrow range of conditions, without any dormancy breaking factors are considered conditionally dormant.

Under a constant temperature regime, all five populations had lower dormancy at 5, 7.5, and 20 C in light than in the dark. However, at the remaining temperatures (10 and 15 C), there was no significant difference between the dark and light treatments. Conversely, under an alternating temperature regime, mean temperatures of 5 and 7.5 C resulted in low seed dormancy in the dark compared to light treatments. However, at 10 and 20 C, dormancy levels did not differ between light and dark treatments; only at 15 C, dormancy rates were lower in light treatments than in dark treatments. When temperature regimes are compared, at sub–optimal temperatures (5 and 7.5 C), these populations had an absolute requirement for light under constant temperature regime; and under a alternating temperatures no such requirement was observed. The ability of light to break seed dormancy has been well studied in relation to mean

temperature and temperature alteration (Taylorson and Hendricks 1972; Pons 1992). Derkx and Karssen (1994) reported that the variation in seed sensitivity to light is an important mechanism that regulates the seasonal dormancy/non–dormancy cycles in Arabidopsis (*Arabidopsis thaliana* [L.] Heynh). It was observed that only at 20 C, both alternating regime, alternating and light combined were effective in breaking dormancy of Turkey, Pink Beauty, Scott, PB–87, and UMan–89. Fluctuating temperatures and light both act as gap–detecting mechanisms and are required to break dormancy and stimulate germination in some species (Thompson and Grime 1983).

Under natural conditions, seeds at greater depths experience dark conditions, cooler temperatures and minor temperature fluctuations, and they tend to be more dormant than seeds planted at shallower depths (Baskin and Baskin 1998). The combination of constant temperature and darkness simulates the field conditions and is often associated with persistent seed banks. Conversely, seeds planted at shallow depths experience alternating temperatures and light conditions and thus should have high germination rates. However, at alternating temperature regime, these populations either showed no requirement for light or no difference in germination rates between light and darkness, demonstrating that temperature fluctuation can substitute for the effect of light. Substituting temperature fluctuation for light to break seed dormancy has been reported in several species (Pons 1992). However, the exceptional high dormancy of alternating temperatures and dark combination at 15 C could not be explained.

Table 4.1 Effects of population, mean temperature, regime, light and their interactions analyzed by multifactor ANOVA. The table shows degrees of freedom (df), F–ratio values and associated probability (P) for main effects and their interactions.

Source of Variation	df	F	P
Population	14	308.00	<.0001
Mean temperature	4	2675.63	<.0001
Regime	1	28.53	<.0001
Light	1	152.25	<.0001
Population × Mean Temperature	56	76.75	<.0001
Population × Regime	14	11.76	<.0001
Population × Light	14	20.11	<.0001
Mean Temperature × Regime	4	374.41	<.0001
Mean Temperature × Light	4	76.79	<.0001
Regime × Light	1	157.00	<.0001
Population × Mean Temperature × Regime	56	13.66	<.0001
Population × Mean Temperature × Light	56	8.94	<.0001
Population × Regime × Light	14	9.73	<.0001
Mean Temperature × Regime × Light	4	375.89	<.0001
Population ×Mean Temperature × Regime × Light	56	9.13	<.0001

***Florist Rose and White Beauty*.** The ornamental populations, Florist Rose and White Beauty, exhibited similar responses to the temperature and light treatments (Figure 4.1). Florist Rose and White Beauty had higher germination rates at 5 and 7.5 C than the other populations. At higher temperatures (15 and 20 C), White Beauty showed less or no germination (< 5%) with no significant difference between any treatment combinations, which was similar to Florist Rose.

Under a constant temperature regime, the lowest dormancy was observed at 5 and 7.5 C, with no significant difference between light (22 and 27%) and dark (12 and 26%) treatments for Florist Rose. A similar pattern was observed for White Beauty. At 10 C, dark treatments resulted in significantly lower seed dormancy 15 and 35% compared to light treatments 56 and 52% for Florist Rose and White Beauty, respectively. Under an alternating temperature regime, Florist Rose showed significant differences in seed dormancy between light and dark treatments at 5 and 7.5 C. However, in the case of White Beauty, no such differences were observed for most of the temperatures.

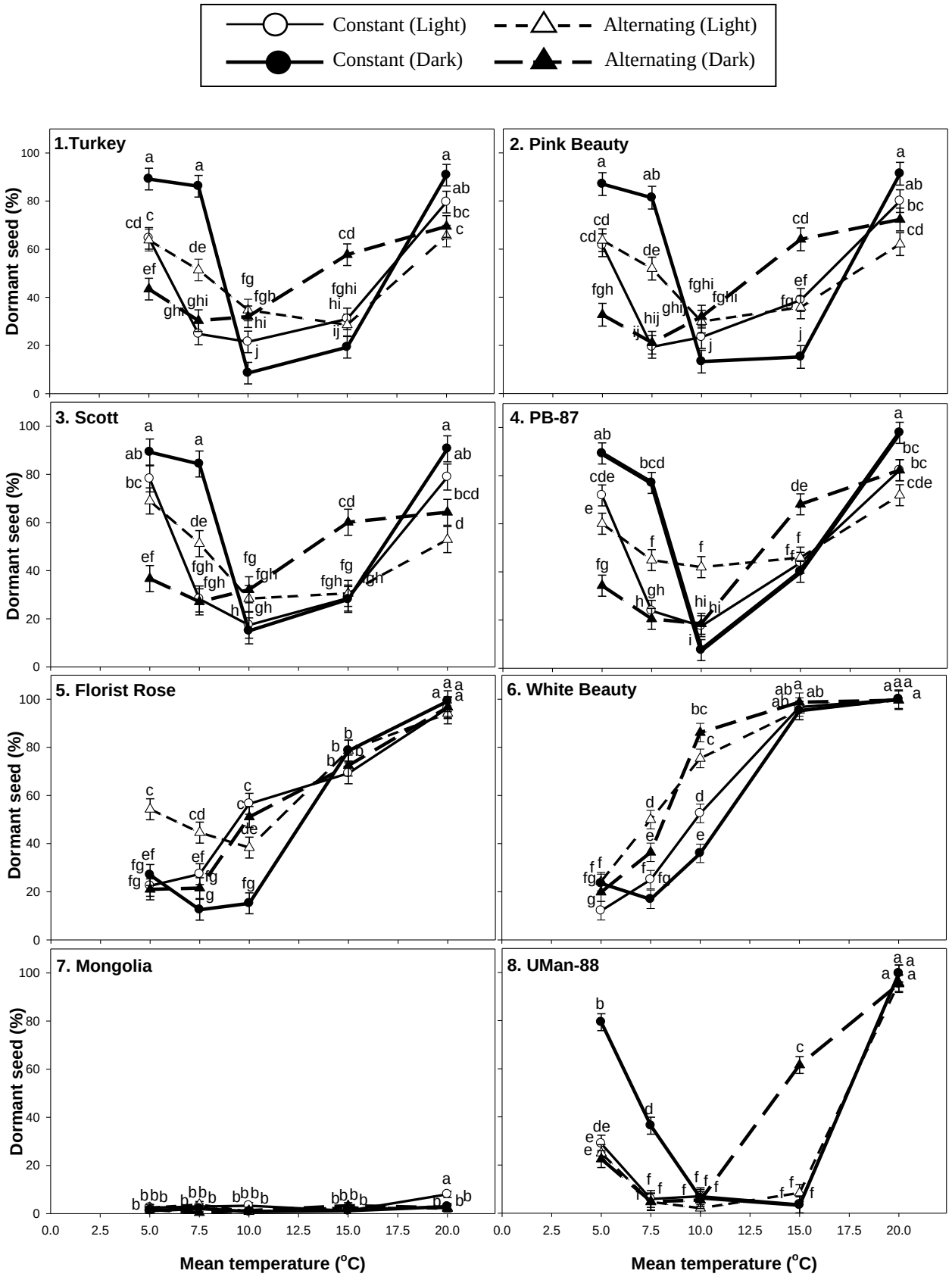
High germination rates at constant temperatures of 5, 7.5 and 10 C in the dark suggest that 5–10 C is the optimal range of temperatures for these populations. Although these populations differ from other cultivated and weedy accessions for ideal temperature for germination, they still exhibit conditional dormancy at 15 and 20 C. In our study, we observed that alternating temperatures and light seem to have little or no effect on the germination of Florist Rose and White Beauty at most of the mean temperatures.

UMan–88, –00, –02, –04 and –05. These weedy populations showed greater germination for the given temperature and light treatments except for Mongolia (Figure 4.1). Among the 5 mean temperatures, 7.5 , 10, and 15 C resulted in the least dormancy for all these populations.

Under constant temperature regime, other than at 5 and 7.5 C, there was no significant difference in germination between light and dark treaments. These populations require light for dormancy loss at constant temperatures of 5 and 7.5 C. Furthermore under alternating temperature regime, the effect of light was not significant on seed dormancy loss except at 15 C. When temperature regimes are compared, alternating regime seems to be more effective in relieving dormancy compared to constant temperatures, with few exceptions.

These weedy populations showed little or no dormancy at 10 and 15 C suggesting the ideal temperature range. At 5 and 7.5 C, these populations either require light or alternating temperature to release seed dormancy. Similar responses were observed for most of the cow cockle populations in the study. Poor germination at low (5 and 7.5 C) and high temperatures (20 C) can prevent germination in hot summers and in autumn. For these populations, the latter is more important to avoid death of the seedlings due to harsh winters of the Canadian prairies. Under natural conditions, the requirement of light or alternating temperatures suggests that even dormancy–broken seeds can form a soil seed bank, associated with low temperature inhibition in winter, to withstand adverse climatic conditions. Furthermore, data from our persistence study showed a long–lived seed bank formation in these populations (data not shown).

Scott Weedy and Saskatoon Weedy. The germination characteristics of these two populations were found to be intermediate between cultivated and weedy populations for the given conditions (Figure 4.1). These two populations had a similar germination pattern compared to the Manitoba populations, for mean temperatures of 10 C and higher (Figure 4.1); and at lower temperatures, were similar to cultivated populations. Similar to most of the populations in the study, highest germinations for Scott Weedy and Saskatoon Weedy were observed at 10 C (97% and 89%, respectively). At mean temperatures of 10 and 15 C, these two populations had low dormancy rates without any dormancy breaking factors. At 5 and 7.5 C, these populations required either alternating temperatures with no light or constant tempratures with light combination to release dormancy.


Constant (Light)
Alternating (Light)
Constant (Dark)
Alternating (Dark)
1.Turkey
2. Pink Beauty
3. Scott
4. PB-87
5. Florist Rose
6. White Beauty
7. Mongolia
8. UMan-88
Dormant seed (%)
Mean temperature (°C)

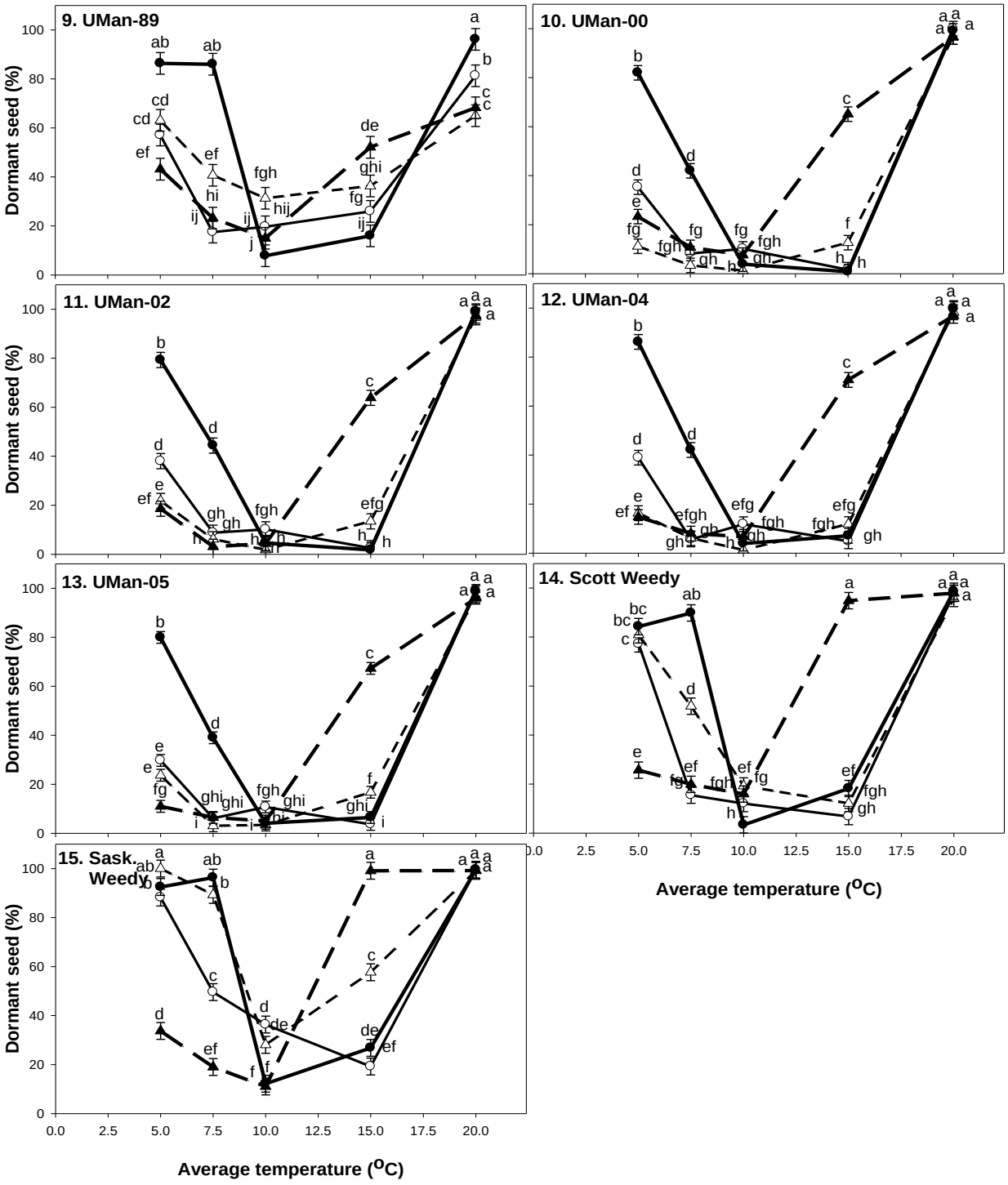


Figure 4.1 Effects of mean temperature, temperature regime (alternating or constant), and light on seed dormancy in fifteen cow cockle populations. Error bars represent the standard errors of least square means. Comparisons are made among treatments; means followed by the same letter are not significantly different at P < 0.05.

4.4.3 Germination Ecology

The "Mongolia" population used in our study had originally been collected from Bulgan somon, Mongolia, and belongs to a desert–steppe ecological zone. A lack of seed dormancy or conditional dormancy occurs in some species in that part of the world (Wesche et al. 2006). The climatic conditions, especially precipitation experienced by Asian deserts could be a possible reason. Although the climate is seasonal, the amount and timing of precipitation is highly unpredictable. In these conditions, non–dormancy would be an important mechanism to take advantage of transiently available moisture and temperature conditions for successful germination and establishment. For example, Tobe et al. (2005) reported lack of dormancy in three desert annuals (*Agriophyllum squarrosum, Bassia dasyphylla* and *Aristida adscensionis*) from China and also suggested that precipitation is the critical factor for seedling emergence in these species. Wesche et al. (2006) examined germination of fresh and frost–treated seeds of 26 species belonging to the desert and mountain steppes of Central Asia, including woody perennials, herbaceous perennials, and short–lived species. Of the eight short–lived species investigated, they found that spineless Russian thistle (*Salsola collina* Pall.) from the Eurasian steppes and semi–deserts was completely non–dormant. Irrespective of growth forms, Wesche et al. (2006) suggested that non–dormancy or conditional dormancy is the most frequent strategy followed by Mongolian dry steppe vegetation.

Similar germination characteristics of Turkey, Pink Beauty, Scott, PB–87 and UMan–89 could not be related to their native habitats conditions, as they have different geographic origins with variable environmental conditions. However, one possible explanation would be the influence of cultivation, since most of these populations were cultivated except UMan–89. The effect of cultivation on seed dormancy and germination has been studied in several species (Qu et al. 2005). During cultivation, the repeated cycles of deliberate sowing and harvesting selects against seed dormancy. This is because the erratic germination of dormant seeds contributes less to the final crop stand (Fuller and Allaby 2009). Additionally, cultivation may also have affected other seed characteristics in relation to their germination behavior, such as seed size, as we observed a larger seed size of these populations (data not shown) compared to other weedy and wild lines. Zareian et al. (2013) reported a significant effect of seed size on germination

characteristics of three wheat (*Triticum aestivum* L.) cultivars both under field and laboratory conditions; and also illustrated a negative correlation between seed size and germination rate.

The dormancy release and germination behaviour of the ornamental populations may correspond to the average spring (6–9 C) and summer (12–16 C) temperatures typical in the United Kingdom, where they orginated. United Kingdom experiences a maritime climate, characterized by mild winters and cool summers. The low temperature germination of these populations is consistent with the origin of these populations. Furthermore, no effect of alternating temperature on the germination may have developed in response to the low variation in seasonal temperature of maritime climates. At certain temperatures, light was observed to have inhibited germination. This can be interpreted as an adaptation strategy to avoid seed germination on the soil surface, common in situations where the soil dries rapidly such as sandy and coastal habitats (Thanos et al. 1991).

All the weedy populations except UMan–89 had a higher germination with most of the treatments indicating that the temperatures used were well suited for these populations. According to Meyer et al. (1990), emergence and survival was higher for seed collections subjected to conditions similar to their parent population. Under natural conditions, the conditional dormancy of these populations may help them to remain ungerminated after dispersal in autumn, thus preventing high seedling mortality under the relatively harsh winters of Canada. This brief period of dormancy (conditional) may also provide enough time for the seeds to get buried and form a persistent seed bank. Seed dormancy acts as a selective advantage in weed species by restricting the germination of viable seed especially in areas with unfavorable seasons (Harlan 1992). This mechanism helps in maintaining the species through generations without extinction. Based on our observations, selection pressures relating to environmental conditions in natural habitats or selection pressures due to frequent disturbances in the agricultural areas or both may have resulted in these weedy populations.

In conclusion, cow cockle exhibits conditional dormancy at maturity, which is variable among the different populations. Although high seed dormancy is undesirable in domesticates, a complete elimination of dormancy may result in problems, such as pre–harvest sprouting especially under cold and humid environments (Gubler et al. 2005). Therefore, if cow cockle

were grown as a crop, the conditional dormancy may not be considered a barrier to domestication and can be viewed as a physiological mechanism to avoid germination at harvest. However, as a weed, conditional dormancy may promote the formation of persistent soil seed banks.

Both temperature and light interacted to relieve cow cockle seed dormancy. However, at optimal mean temperatures, light or temperature alternation had no effect on cow cockle seed germination. At sub–optimal (5 and 7.5 C) and supra–optimal conditions (20 C), alternating temperatures had major impact on the germination of conditionally–dormant cow cockle seed, followed by light. Furthermore, with these conditions, the requirement of alternating temperatures to promote seed germination could be partially replaced by light in cow cockle.

Although seed dormancy is a qualitative trait, inter–population germination differences may be generated by prevailing environments during seed maturation and/or due to genetic differentiation resulting from previous selection pressures (Meyer et al. 1990). Evidence from several studies demonstrates a possible selection pressure of the species habitat conditions on germination strategies (Baskin and Baskin 1988). Similarly, the differential germination and dormancy patterns among cow cockle populations may have evolved in response to their native habitats or evolved to adapt to exotic habitats (Nikolaeva 2004). For example, variation in seed dormancy and germination traits was reported in crofton weed (*Ageratina adenophora* [Spreng.] R. M. King & H. Robinson) grown at different elevations (Li and Feng 2009). Sosebee and Wester (1995) defined ecotypic variation as "genetically based variation within a species that is correlated to habitat or environment". Since these populations originated from different parts of the world, the differences in soil, water, light, temperature and other environmental conditions may have contributed to their variable germination strategies. Furthermore, with the existing variability in environmental conditions in different habitats, the degree of risk associated for germination also varies. Therefore the dormancy release and germination patterns observed in these populations probably evolved as they adapted to their local environments.

PROLOGUE (CHAPTER 5)

It is generally believed that seed dormancy aids in persistent seed bank formation. Hence, the conditional dormancy of cow cockle populations (Chapter 4) may contribute to high seed persistence. Persistent seed bank formation is one of the essential characteristics in many wild and weedy plants, as it maintains seed supply in the soil for extended periods which buffers against extinction in unfavorable years (Fenner and Thompson 2005). Most domesticated crops have transient seed banks, as high seed persistence in crop plants lead to problems such as volunteers in subsequent crops as well as erratic germination (Gressel 2005). High seed persistence in wild or weedy species altered during the process of domestication, as most cultivated species cannot persist in natural habitats (Pessel et al., 2001). Therefore, a study was initiated to determine whether cow cockle is persistent and forms a seed bank (Chapter 5). As part of the study, two comparative experiments (field and laboratory) were conducted from 2008 through 2011. Results for field emergence and the residual seed bank revealed a greater longevity of weedy seeds than those of the cultivated population at all locations. Despite the differences in seed persistence between the populations, considerable numbers of seed of both weedy and cultivated lines were recovered from the soil seed bank at the end of the study. Similar results were also observed in the laboratory study. This depicts that cow cockle is persistent and can form a reasonably long–term seed bank. The substantial seed persistence in cow cockle may pose some concerns on production of cow cockle as a crop for the Canadian prairies. Agronomic practices may have to be optimized to tackle the seed persistence problem in cow cockle; such as harvesting to reduce the combine losses and pre–seed spring tillage followed by in–crop herbicide control.

5.0 EVALUATION OF SEED PERSISTENCE IN COW COCKLE (*Vaccaria hispanica* [P. Mill.] Rauschert), A POTENTIAL ALTERNATE CROP

5.1 ABSTRACT

Cow cockle (*Vaccaria hispanica* [P. Mill.] Rauschert) is an introduced summer annual weed in North America. It has been investigated as a potential crop for the Canadian prairies. Seed persistence contributes to volunteers in subsequent crops; therefore, it is undesirable in this prospective crop. To determine whether cow cockle is persistent and forms a seed bank, available germplasm was evaluated in two comparative experiments (field and laboratory), conducted from 2008 through 2011. The field study was conducted at three locations across Western Canada to investigate the field persistence and residual seed bank of a cultivated and a weedy cow cockle population under spring–tilled and zero–tillage systems. Seedling recruitment was monitored for three years and the residual seed bank was sampled at the end of the experiment. To study seed persistence in the lab, a controlled aging test was performed for 15 cow cockle populations at elevated temperature and relative humidity (45 C and 60% RH) with periodical sampling for germination and viability. The time taken in aging days for viability to be reduced to 50% (p_{50}) was used to determine relative seed longevity for the populations. In the field study, the 3yr decline rate for emergence for both the populations was exponential. The weedy population had higher seedling emergence at two of three locations and a larger residual seed bank at all locations. Spring tillage promoted greater seedling recruitment in both populations compared to zero–tilled treatments. However, populations did not differ in the size of the seed bank under spring tillage, whereas a larger soil seed bank of weedy compared to cultivated population was observed in no–till treatments. In the laboratory aging study, the cow cockle populations showed considerable differences in seed longevity, as p_{50} varied from 25 to 77 d. The seed longevity from the aging experiment was associated with seed dormancy and germination characteristics of the populations. Although the cultivated population showed less seed persistence compared to the weedy population, both field and laboratory studies suggested that a considerable amount of seed of the cultivated population persists in the soil after 3 years and may pose volunteer problems in subsequent crops. Agronomic practices that are focused on reducing the harvest losses and pre–seed spring tillage followed by in–crop herbicide control may have to be optimized before cow cockle can be grown as a crop.

5.2 INTRODUCTION

Seeds may die in the soil because of pathogen attack or predation (Clark and Wilson 2003); suicidal germination in the deeper layers of soil (Traba et al. 2004; James et al. 2011) or aging (Rice and Dyer 2001). Most general classification categorizes soil seed banks into either transient (< 1 year) or persistent (> 1 year) based on the longevity of the seeds in the seed bank (Thompson and Grime 1979).

Persistent seed bank formation is one of the essential characteristics in many wild and weedy plants, as it maintains seed supply in the soil for extended periods which buffers against local extinction in unfavorable years (Fenner and Thompson 2005). High seed persistence in crop plants lead to problems such as volunteers in subsequent crops as well as erratic germination (Gressel 2005). Seed bank persistence of crops may also create new environmental and crop–weed management risks in agricultural systems (Smyth et al. 2002). High seed persistence in wild or weedy species is altered during the process of domestication, as most cultivated species cannot persist in natural habitats (Pessel et al. 2001). The seeds of highly domesticated crops such as maize (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) rarely persist over a year. In contrast, many wild or weed plants form long–lived seed banks (Holm et al. 1997; Khan et al. 1997; Chadoeuf et al. 1998). Hails et al. (1997) reported low seed persistence in cultivated canola populations (*Brassica napus* subsp. *oleifera* DC Metzger) when compared to their weedy relative, wild mustard (*Sinapis arvensis* L.). They found that seeds of wild mustard survived to a greater extent in both first and second years of study (average 60% and 32.5%) when compared to cultivated canola populations (average 1.2% and 2%) at all three locations. Similarly, Noldin et al. (2006) reported higher seed persistence in weedy red rice ecotypes when compared to the domesticated genotypes.

Field persistence experiments incorporate environmental factors (biotic and abiotic) and therefore are considered reliable and informative. However, they are time–consuming, financially demanding and ineffective in large–scale studies (Ter Heerdt et al. 1996; Ishikawa–Goto and Tsuyuzaki 2004). Alternatively, laboratory studies on seed longevity are simple and inexpensive. Although this method fails to simulate the ecological complexity of the field, it allows differences in seed persistence to be quantified under controlled conditions. Laboratory

approaches that utilize seed (physical and chemical) characteristics to estimate seed persistence have been proposed by several researchers (Daws et al. 2006). However, a lack of correlation between laboratory and field seed longevity highlights the importance of *in situ* conditions and on seed survival in the soil. Priestley et al. (1985) reported a significant correlation between p_{50} values (laboratory) and seed longevity index (field), with 52% of field variability in seed longevity being explained by the laboratory study. Recent studies suggested a positive correlation between controlled ageing conditions (45 C and 60% RH) and field seed persistence (Long et al. 2008).

Cow cockle (*Vaccaria hispanica* [P. Mill.] Rauschert) is an introduced summer annual in Canada and a minor weed especially in fine textured soils (Balsevich 2008). Cow cockle is being considered for commercial crop production because of seed compositions including ultrafine starch (Biliaderis et al. 1993), cytotoxic saponins (Balsevich et al. 2006) and their potential applications in the cosmetic and medicinal industries. Furthermore, favorable agronomic characteristics including high seed yield indicate the potential of cultivating cow cockle as a crop (Goering et al. 1966). However, cow cockle has a long history of being a weed in Canada and therefore it is essential to understand the weediness potential of this species before initiating commercial production. Seed bank persistence is a common trait of most weeds however it has not been studied in cow cockle. Accordingly, the purpose of the study was to assess the weediness potential of cow cockle with respect to its seed persistence. The objectives of the study include evaluation of available germplasm to determine if cow cockle is persistent under conditions of cultivation and forms a seed bank. The present study includes two experiments aiming to compare seed longevity among populations of cow cockle under both field and laboratory conditions.

5.3 MATERIALS AND METHODS

5.3.1 Field Study

5.3.1.1 Location and Experimental description

The field study was initiated in the fall of 2008. It was conducted for three years in the Northern Great Plains (2009, 2010 and 2011) at three different locations; the Saskatoon, SK (52^{o}

N 106^{o} W), Scott, SK (52^{o}36’ N, 108^{o}84’ W), and Lethbridge, AB (49^{o}41’ N, 112^{o}37’ W). The treatments were allocated in a 2 x 2 factorial design with seed source (cultivated and weedy cow cockle) as one factor and tillage system (spring pre–seed tillage and zero tillage) as the second factor. The treatments were replicated 6 times in a randomized complete block design. The seed for the above treatments was obtained from the Scott Research Farm. The cultivated population used in the study was “Scott”, a selection from the Scott Research Farm in their weed control studies. The weedy cow cockle is the “Scott Weedy” population which was collected by staff from the Scott Research Farm, from a lentil field located 150 km south of Scott following lentil harvest in 2007.

Seed was broadcasted into 6 x 6 m plots at a rate of 1200 seeds m^{-2} in mid to late October, 2008. These rates were based on estimates of harvest losses from field scale plots grown at the Scott Research Station. Prior to seeding, 20 soil cores, 7.5 diameter at a depth of 10 cm were taken across the experimental area and the soil samples were germinated under controlled conditions to quantify the background cow cockle seed; which was observed to be insignificant (data not shown). Cropping sequence for this three–year period was spring wheat at 250 seeds m^{-2} in 2009, glyphosate–resistant canola at 150 seeds m^{-2} in 2010 and spring barley at 250 seeds m^{-2} in 2011. For tillage treatments, zero till plots had a pre–seed burn–off application of glyphosate at 450 g a.i ha^{-1}, while the spring pre–seed tillage plots received one cultivator pass prior to seeding. In–crop weed control consisted of thifensulfuron/tribenuron at 15 g a.i ha^{-1}, glyphosate at 450 g a.i ha^{-1} and bromoxynil/MCPA ester at 560 g a.i ha^{-1} for the three years sequentially.

5.3.1.2 Data Collection and Analysis

5.3.1.2.1 Emergence Counts

Cow cockle counts (weedy and cultivated) were recorded four times during the growing season in 15 random 0.25 m^2 quadrats per plot, when weed populations were low the entire plot area was counted. Plants were counted just prior to spring burn–off or spring cultivation; in–crop prior to post–emergence spraying; three weeks after in–crop herbicide application; and post–harvest. Plants surviving the post–emergence application were removed after counting to avoid fresh seed return to the seed bank. Dates of main cultural practices and observations in the fields are presented in Table 5.1.

5.3.1.2.2 Soil Sampling and Processing

At the end of the three years, an intensive soil sampling was conducted to estimate viable seed in the soil seed bank. Twenty random soil cores, 7.5 cm diameter and 10 cm deep were obtained from each plot at all the locations. Samples were air–dried immediately to avoid germination and stored until cleaning. The soil samples were broken coarsely and passed through a custom–made belt thresher adjusted for the safe passage of seeds. The crushed samples were passed through a Carter Day dockage tester (CEA. Simon–Day LTD.) using a sieve combination (1mm/2.5mm) to remove particles smaller or larger particles than seed. The resulting soil seed sample was soaked in water for a brief period to facilitate separation of seed from stones. These samples were oven–dried and stones were removed by using the rolling movement of seed followed by hand picking. A crush test (Sawma and Mohler 2002) was used to determine the viability of the cleaned seed.

Table 5.1 Dates of main cultural practices and observations in the fields where seeds of cultivated and weedy populations were buried at Lethbridge, Scott and Saskatoon

Year	Activity	Lethbridge	Scott	Saskatoon
2009	Early spring emergence counts	May 21st & 22nd	May 20th	May 19th
	Pre–seed Burn–off	May 20th	May 25th	May 23rd
	Pre–seed tillage	May 25th	May 25th	May 24th
	Seeding (Spring Wheat)	May 27th	May 26th	May 27th
	Pre–spray emergence counts	June 24th	June 16th	July 2nd
	In–crop spraying	June 24th	June 17th	July 5th
	Post–spray emergence counts	July 22nd	July 31st	Aug 28th
	Post–harvest counts	Nov 3rd	Oct 13th	Oct 23rd
2010	Early spring emergence counts	May 26th	May 7th	May 17th
	Pre–seed Burn–off	May 27th & July 28th	May 12th	May 20th
	Pre–seed tillage	July 9th	May 7th	May 22nd
	Seeding (Canola)	Aug 9th	May 10th	May 27th
	Pre–spray emergence counts	Aug 31st	June 7th	June 15th
	In–crop spraying	Sept 2nd	June 12th	June 22nd
	Post–spray emergence counts	Oct 7th	July 9th	July 21st
	Post–harvest counts	Nov 1st	Oct 6th	Oct 1st
2011	Early spring emergence counts	June 14th	May 20th	May 25th
	Pre–seed Burn–off	June 14th	May 24th	May 29th
	Pre–seed tillage	June 28th	May 24th	May 30th
	Seeding (Spring Barley)	June 29th	May 25th	June 2nd
	Pre–spray emergence counts	July 18th	June 13th	June 15th
	In–crop spraying	July 19th	June 13th & 24th	June 18th
	Post–spray emergence counts	August 9th	June 30th	July 18th
	Post–harvest counts	Sept 27th	Sept 12th	Sept 15th
	Soil Sampling	Oct 4th	Sept 29th	Sept 23rd & 26th

5.3.1.2.3 Data Analysis:

Data analysis was done separately for the three locations, as there was a significant location or location by treatment effect (Table 5.2). The data met the assumptions of normality and homogeneity of variances for Analysis of Variance (ANOVA). ANOVA was performed using SAS mixed models (SAS Institute Inc., 2008) with repeated measures. Population, tillage, month and year were considered fixed effects, while block, location and location by fixed effects interactions were considered as random. Plant counts were considered repeated within the location for three years with spatial power covariance structure. Depending on the location by fixed effect interaction ($P < 0.05$), it was determined whether the data analysis could be combined or by individual location. To obtain the best simple model, model simplification was carried out by removing non–significant covariant parameters based on the Akaike's Information Criterion (AIC) values (Littell et al. 2005). When the ANOVA detected a significant main effect or interaction, Fisher's protected LSD tests were used for mean separation. The final seed bank measure was analyzed using the mixed procedure of SAS. The main factors population and tillage were taken as fixed and block as random factor, which is nested within location.

Table 5.2 Analysis of variance for seedling emergence of cow cockle within each location as affected by time, population and tillage assessed at Lethbridge, Saskatoon and Scott in 2009, 2010 and 2011. Within each location, the table presents degrees of freedom (df) and associated probability (P) for three–way and four–way interactions of fixed factors

Source of Variation		df	P
Random factors			
Block		5	0.2790
Month x Location		6	0.0858
Population x Month x Location		6	0.1167
Population x Month x Year x Location		12	0.0178
Tillage x Month x Year x Location		12	<.0008
Fixed factors			
Population x Tillage x Month	Lethbridge	3	0.9694
Population x Tillage x Year		2	0.6166
Population x Month x Year		6	<.0001
Tillage x Month x Year		6	<.0001
Population x Tillage x Month x Year		6	0.9915
Population x Tillage x Month	Saskatoon	3	0.9311
Population x Tillage x Year		2	0.8109
Population x Month x Year		6	<.0001
Tillage x Month x Year		6	<.0001
Population x Tillage x Month x Year		6	0.834
Population x Tillage x Month	Scott	3	0.9736
Population x Tillage x Year		2	0.3712
Population x Month x Year		6	<.0001
Tillage x Month x Year		6	<.0001
Population x Tillage x Month x Year		6	0.4899

† Random factors are based on model simplification.

5.3.2 Laboratory study

5.3.2.1 Seed Source

The laboratory study was initiated in March, 2012. The study material comprised of 15 cow cockle populations from different parts of world (Table 5.3; described in Chapter 4). The seed material for the experiment was obtained from crop grown in a separate experiment in 2011 at the Kernen Crop Research Farm, Saskatoon, SK, Canada (59^{o} 09' N, long 106^{o} 33' W). The plots were harvested at maturity with a small plot harvester leaving two rows on either side. Seed was cleaned with a Carter Day dockage tester and a seed blower (Fasco industries, D127). The cleaned seeds were air–dried at room temperature (20 – 25 C) for 10 days to reduce the seed moisture for safe storage. Seed samples weighing ≥100 grams were randomly sampled for each population and stored at low temperature and humidity storage environment (15 C and 20% RH) until the initiation of the present experiment.

5.3.2.2 Germination Test

Control aging tests require an initial germination of greater than 85%. Germination tests were performed by placing seeds in 9–cm petri–dishes lined with double–layered filter paper that had been moistened with 5 ml of distilled water. Three replicates of 50 seeds per population were germinated under ideal temperature and photoperiod conditions, which were established in our previous dormancy experiment (10 C and 12h/12h dark/light; Chapter 3). The germination trays were sealed with clear plastic bags to prevent moisture loss. Germination counts were recorded periodically for up to 30 d. Seeds with radical protrusion were considered germinated (≥ 2 mm). After the germination period, viability test was conducted for un–germinated seeds and the unviable seeds were deducted from the total seed count. Final germination was adjusted based on initial measure of viable seeds.

5.3.2.3 Controlled Aging Test

The relative longevity of seed of each population was determined by following the protocol of Probert et al. (2009). In this method, different concentrations of lithium chloride were utilized to obtain desired relative humidity conditions (47% and 60%). Ten samples of 50 seeds of each population were placed in open glass vials over a 47.0 ± 1.5% RH lithium chloride

solution (370 g L^{-1} H_2O) within a sealed container at 20 ± 1 C for 14 d to rehydrate the seeds. After the re–hydration period, the seeds were transferred to a second container over a solution of lithium chloride at 60% RH (300 g L^{-1} H_2O) in an oven set at 45 C. Temperature and RH were monitored and maintained throughout the experiment with traceable humidity/thermometer remote sensor modules placed within the containers. Ten replicates of 50 seeds per population were used for the test. Samples were removed after 1, 2, 5, 9, 20, 30, 50, 75, 100 and 125 d and tested for viability under previously found optimal germination conditions for 30 d. Normal germination (percentage) were plotted against time in aging (days) for each population. Three–parameter log logistic curves were fitted to the data.

$$\text{Germination (\%)} = \alpha / \{1+\exp [\beta \log (t) - \log p_{50}]\} \quad (5.1)$$

where α is fitted initial germination (percentage), β is the rate of viability loss in the rapidly declining section of the curve and t is the accumulated time in the CAT (in days). Germination data were tested for significance using nonlinear regression analysis of curves and model parameters using the *drm* package in R (Version 2.10, http://www.R–project.org). The populations were grouped such that there is no significant difference between the curves of the populations within the group. This analysis was done by comparing seed survival curves of each population with a common curve of all the populations in the group. Student–Newman–Keuls post–hoc test for one–way ANOVA was used to compare the mean p_{50} values of the groups.

Table 5.3 Accessions of *Vaccaria hispanica* (P. Mill.) Rauschert evaluated in controlled aging study. Thousand seed weights (TSW) and time to 50% seed viability loss (p_{50}) in days for all 15 populations.

Accession name	Origin	Putative status	TSW (g)	Mean p_{50} (d)
Pink Beauty	Finland	Cultivated	7.49	62.2
Turkey	Turkey	Cultivated	7.26	64.2
Scott*	Canada	Cultivated	7.22	58.6
PB–87	Finland	Cultivated	6.89	59.4
Florist Rose	United Kingdom	Cultivated (ornamental)	5.58	77.4
White Beauty	United Kingdom	Cultivated (ornamental)	5.48	69.8
Mongolia	Mongolia	Wild	5.73	24.8
UMAN–88	Canada	Weedy	3.46	41.5
UMAN–89	Canada	Weedy	7.35	62.0
UMAN–00	Canada	Weedy	3.38	44.3
UMAN–02	Canada	Weedy	3.39	41.1
UMAN–04	Canada	Weedy	3.43	40.8
UMAN–05	Canada	Weedy	3.68	39.3
Scott Weedy*	Canada	Weedy	3.31	47.0
Saskatoon Weedy	Canada	Weedy	3.29	52.5

* Populations evaluated in the field persistence study.

5.4 RESULTS

5.4.1 Field Study

The effect of location on emergence was significant (P<.0001; Table 5.2). Hence the analysis was conducted separately for each location. Within location, most of the variation in emergence was due to population or tillage but not their interaction (Table 5.2). Other factors such as month and year interacted with tillage or population to affect emergence within locations (P<.0001).

5.4.1.1 Population

Emergence patterns of cow cockle differed among the three locations (Table 5.2). Additionally, seedling emergence of each population differed (P<0.001) within each location (Table 5.2). The emergence of the weedy population was greater than the cultivated population at Lethbridge and Scott, but not at Saskatoon (Figure 5.1). At Lethbridge, the weedy population consistently emerged in greater numbers than the cultivated population in all three years. Overall the weedy population showed 44% greater emergence than the cultivated line at this location. Similar results were also observed at Scott for the first two years of seed burial (Figure 5.1). In contrast, seedling emergence of the cultivated population was 63% greater than the weedy population by the end of three years at Saskatoon (Figure 5.1).

The timing of peak seedling emergence also differed among locations. In 2009, Lethbridge experienced relatively warmer temperatures and moderate rainfall in May when compared to other locations (Table 5.4), this may have contributed to the greater emergence of cow cockle. At the other locations, temperatures and rainfall during the summer months resulted in peak emergence (Figure 5.1). In the remaining years (2010 and 2011), the difference in timing of peak emergence between the locations may have resulted due to the variability in date of counting (Table 5.1).

Table 5.4 Monthly mean temperature (C) and rainfall (mm) for Lethbridge, Scott and Saskatoon from May until October in 2009, 2010 and 2011 and the climate normals (30–yr average).

		Temperature				Rainfall			
Location	Month	2009	2010	2011	Normal	2009	2010	2011	Normal
		(C)				(mm)			
Lethbridge	May	10.7	7.8	9.4	11.4	19.0	9.4	90.4	46.2
	June	14.1	14.2	13.7	15.6	38.8	109.6	81.4	53.0
	July	17.2	16.9	17.6	18.2	51.6	45.4	51.2	37.2
	August	16.5	15.8	17.8	17.7	73.0	68.8	37.2	47.4
	September	15.8	10.6	14.9	12.3	6.6	76.0	10.6	37.3
	October	3.0	8.4	7.3	7.2	13.0	4.0	75.0	9.0
	Total	–	–	–	–	202	313.2	345.8	230.1
Scott	May	8.7	8.8	10.1	10.9	19.0	121.4	30.8	34.9
	June	14.0	14.9	14.4	15.2	30.4	147.2	190.2	62.5
	July	15.8	16.5	17.0	17.0	74.6	122.4	76.2	70.9
	August	15.4	15.2	16.3	16.3	57.6	61.8	51.8	43.1
	September	14.8	9.5	13.7	10.4	19.4	44.2	3.8	29.1
	October	1.2	5.8	5.6	3.8	36.5	18.3	9.1	9.9
	Total	–	–	–	–	237.5	515.3	361.9	250.4
Saskatoon	May	9.2	9.9	11.3	11.5	5.5	127.5	M	46.8
	June	15.4	15.8	15.8	16.0	81.5	180.5	M	61.1
	July	16.3	17.9	19	18.2	58.5	69.5	M	60.1
	August	16.0	16.3	17.8	17.3	90.5	M	M	38.8
	September	16.0	10.4	14.4	11.2	31.5	M	M	29.0
	October	1.5	6.1	6.4	4.5	43.2	M	M	8.6
	Total	–	–	–	–	310.7	M	M	244.4

† 1970–2000 Canadian Climate Normals obtained from Environment Canada (2013).
M – denotes missing weather data.

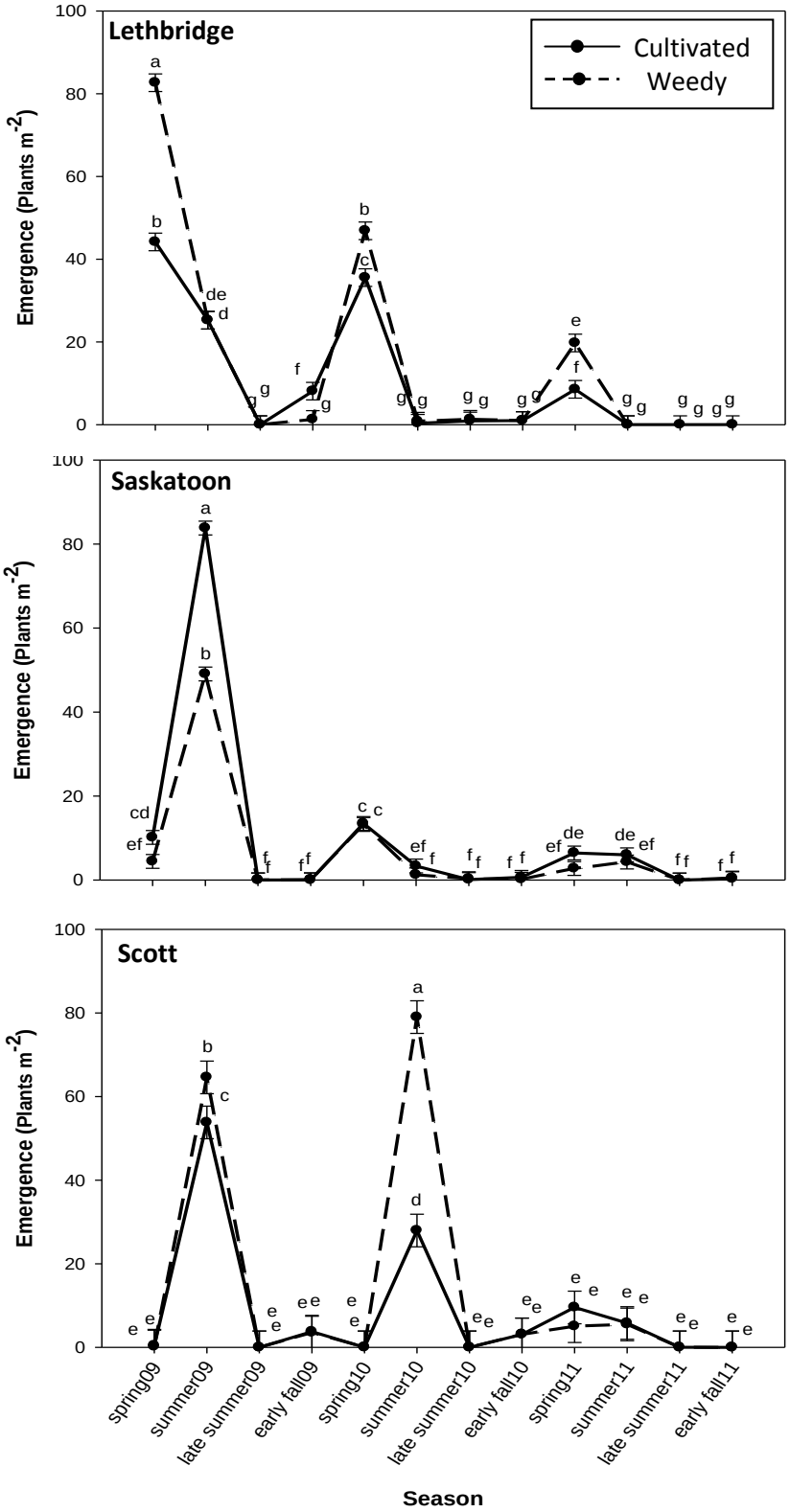


Figure 5.1 Seedling emergences averaged over tillage system of both populations over the three years for three locations. Error bars represent the standard errors of least square means. Comparisons are made between the populations; means followed by the same letter are not significantly different at P < 0.05

5.4.1.2 Tillage

Within each location, tillage affected the timing of cow cockle emergence (Table 5.2). In general, spring tillage resulted in greater summer emergence during the first summer with the exception of Lethbridge (Figure 5.2). At Lethbridge, there were inconsistent differences between the tillage systems for cow cockle emergence during the first year of seed burial; where cow cockle in zero–till treatments had 14% greater emergence than tilled treatments in the spring. Conversely, during the summer, tillage promoted more seedling emergence when compared to zero–tillage (Figure 5.2). In 2010, emergence in the spring was 21% greater in tilled treatments relative to zero–tilled treatments.

At Saskatoon, the effect of spring tillage was prominent during the first two years; however it varied with the season (Figure 5.2). Tillage promoted 96% greater emergence over zero tillage in the summer of 2009. Similar to Lethbridge, in the spring of 2010, the emergence in tilled treatments was twice that of zero tillage treatments and no emergence differences were observed in the summer.

At Scott, total emergence was similar to the other locations and was higher in tilled treatments than in zero tilled treatments (Figure 5.2). However, the peak emergence for the tillage treatments varied between years. Tillage promoted a very high emergence (110 plants m^{-2}) when compared to zero tillage (8 plants m^{-2}) in 2009; whereas in 2010, high seedling recruitment occurred in zero tilled treatments (61%) compared to the tilled treatments.

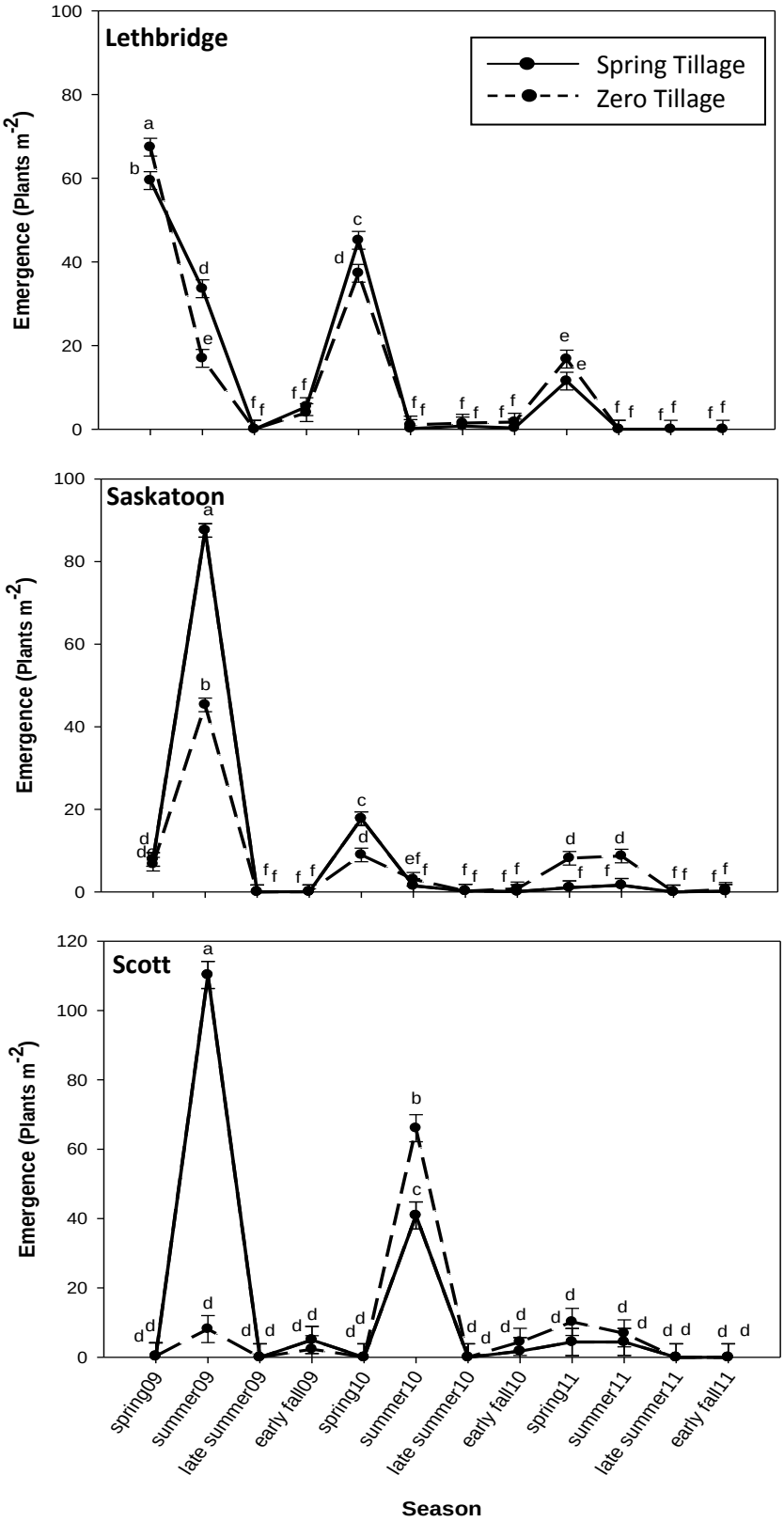


Figure 5.2 Seedling emergences of spring and zero–tillage treatments over the three years for three locations. Error bars represent the standard errors of least square means. Comparisons are made between the tillage systems; means followed by the same letter are not significantly different at P < 0.05.

5.4.1.3 Residual seed bank

The residual soil seed bank differed significantly between the population and tillage treatments. However, this was not consistent over locations (Table 5.5). Regardless of location, population had a significant effect ($P<0.05$) on the residual seed bank of cow cockle. Overall, the weedy population had a more persistent seed bank than the cultivated population (Figure 5.3). However, at Saskatoon and Scott, tillage interacted with population to affect the cow cockle seed bank formation. We found no differences in the size of the soil seed bank between the populations under spring tillage (Figure 5.3). However, in no till conditions, a two to four–fold larger soil seed bank of the weedy population compared to the cultivated population was observed at Saskatoon and Scott, respectively (Figure 5.3).

5.4.2 Laboratory Study

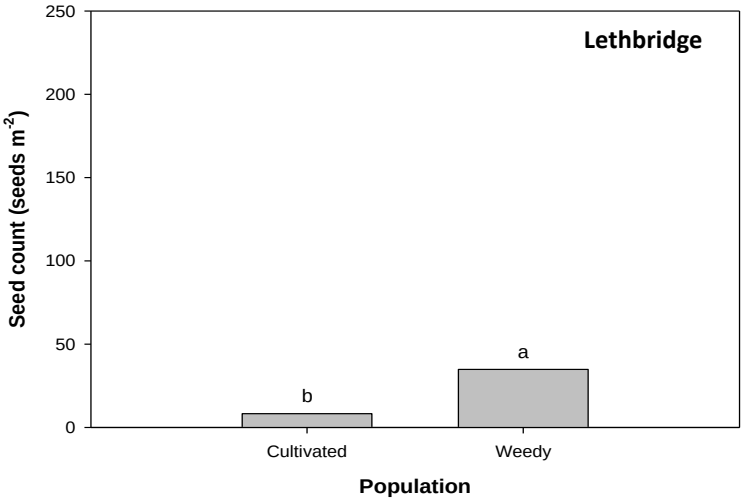
5.4.2.1 Controlled Aging Test

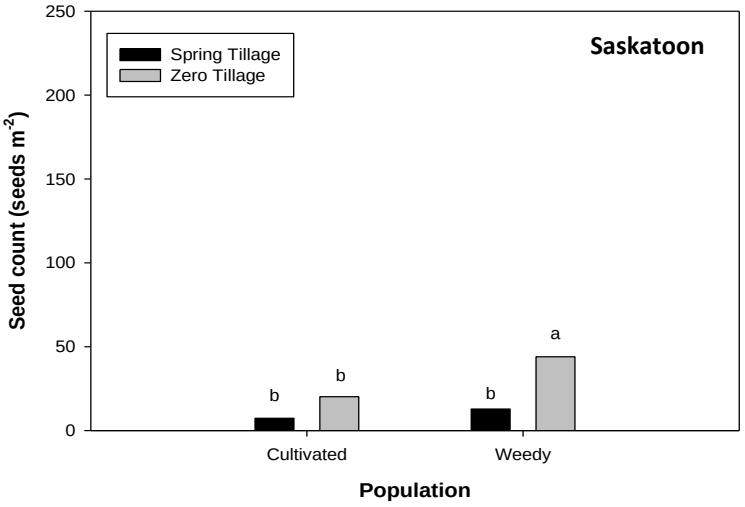
The seed viability declined in a sigmoidal fashion with increasing duration of the aging treatment for all 15 populations (Figure 5.4). Differences were observed for individual populations in seed longevity (Figure 5.5), as measured by the time to 50% mortality in the seed (p_{50}); however, there were similarities among the populations, as four types of seed survival curves were observed (Figure 5.4). The populations were grouped such that there is no difference between the curves of the populations within the group and significant difference between the groups (Figure 5.4).

Irrespective of domestication status (putative), longer p_{50} values were observed for cultivated and cultivated ornamentals than wild and weedy populations (Figure 5.5). The seed longevity (p_{50}) differed significantly among the groups (P <0.0001; Figure 5.5). The p_{50} ranged between 25 d for group 1 to 77 d for group 4. The seeds of Mongolia were significantly shorter–lived when compared to the rest of the populations as it showed a steep decline in viability (Figure 5.4). Mongolia was the only population in Group 1. With the exception of UMan–89, all other weedy populations from Manitoba were classed as Group 2 with a mean p_{50} of 41 d. Group 3 include Pink beauty, Turkey, Scott, PB–87, UMan–89, Scott Weedy and Saskatoon Weedy. The seed survival curves were similar among these populations, with their p_{50} values nearly twice that of Mongolia (Figure 5.5). The seeds of Florist Rose and White Beauty survived longer than all other populations and formed the final group.

Table 5.5 Analysis of variance for residual seed bank of cow cockle within each location as affected by population, tillage and their interactions assessed in 2011. The table shows degrees of freedom (df), F–ratio values and associated probability (P).

Location	Source of Variation	df	F	P
Lethbridge	Population	1	18.05	0.0004
	Tillage	1	2.60	0.1228
	Population*Tillage	1	0.02	0.885
Saskatoon	Population	1	6.91	0.019
	Tillage	1	15.54	0.0013
	Population*Tillage	1	2.70	0.0513
Scott	Population	1	23.56	0.0002
	Tillage	1	65.45	<.0001
	Population*Tillage	1	21.37	0.0003





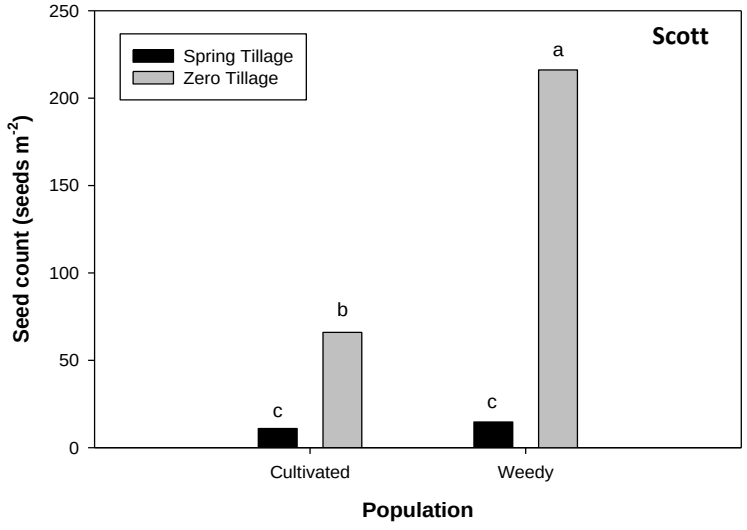


Figure 5.3 Number of viable seeds present in soil at the end of third year as affected by population and tillage for three locations. Comparisons are made between the populations; means followed by the same letter are not significantly different at P < 0.05.

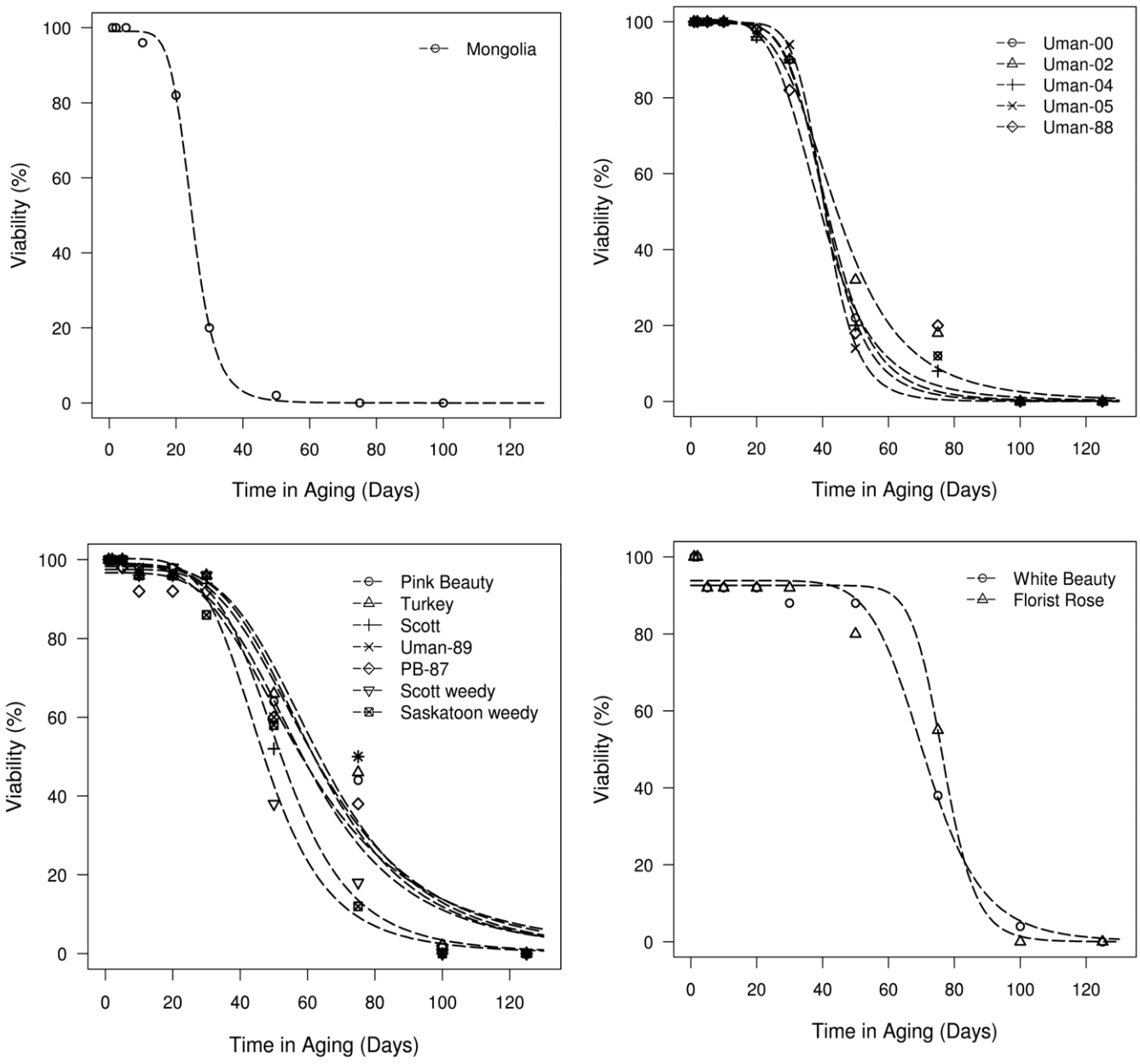


Figure 5.4 Seed survival curves fitted by three parameter log–logistic model for 15 populations in the controlled aging study. The populations were grouped such that there is no significant difference between the curves within the group.

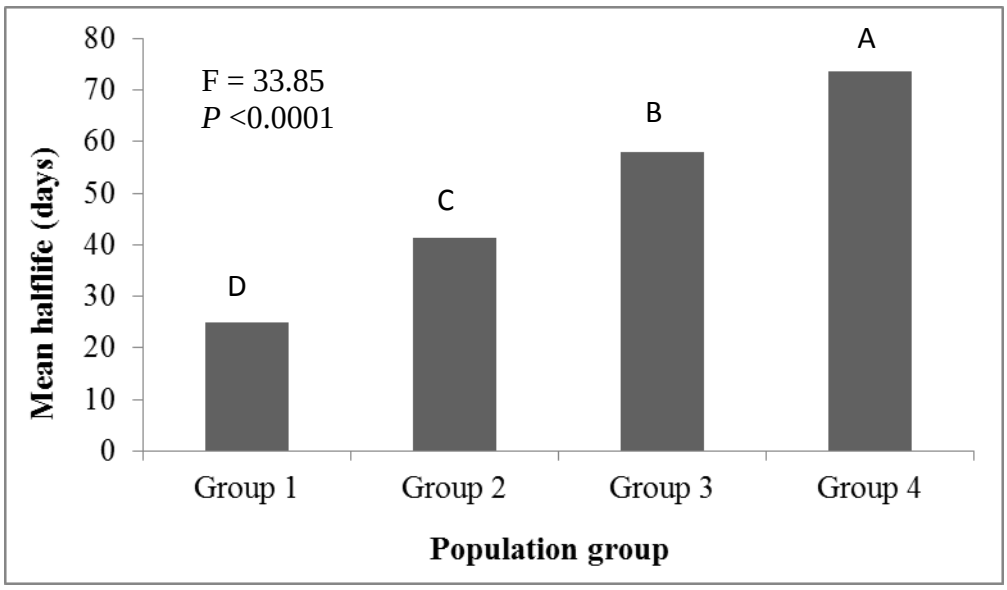


Figure 5.5 Mean half–life (p_{50}) of the population groups. Comparisons are made between the groups; means followed by the same letter are not significantly different at P <0 .05. Group 1, Mongolia; Group 2, UMan–88, UMan–00, UMan–02, UMan–04, UMan–05; Group 3, Pink Beauty, Turkey, Scott, UMan–89, PB–87, Scott Weedy, Saskatoon Weedy; Group 4, White Beauty, Florist Rose.

5.5 DISCUSSION

In the field study, the emergence of weedy population was consistently higher than cultivated population throughout the study period, except at Saskatoon. In addition to a higher seedling emergence, substantial numbers of seed were recovered from the soil seed bank for the weedy population after the three years of study (Figure 5.3); suggesting greater longevity of weedy seeds compared to the cultivated population at all locations. In general, most weed species develop persistent seed banks when compared to domesticated plants. High seed persistence in weedy or wild populations acts as a buffer against reproductive failure and local extinction (Teo–Sherrell et al. 1996). For example, in *Sorghum bicolor* L., Adugna (2013) reported differences in seed longevity between crop and wild populations. The crop seeds were depleted within the first six months. In contrast, the wild populations showed a mean viability of 1.24% after 24 months of seed burial. Similar results were also reported by Chadoeuf et al. (1998) in *Brassica* species; they observed a slower rate of viability loss in weedy hoary mustard (*Hirschfeldia incana* L.) compared to cultivated rape (*Brassica napus* L.) and the inter–generic hybrid between rape and hoary mustard. However, in the present study, the differences in overall seed persistence between cultivated and weedy population are not great, suggesting that the cultivated population, Scott is not fully domesticated.

The differential seed longevity in the field for both populations could be attributed to different causes. First, it may be due to inherent capacity of seed to resist seed deterioration, which is often associated with genetic makeup within the seed (Harrison et al. 2003; Walters et al. 2005). Furthermore, differences in selection pressure on these populations during their adaptation to a specific environment may have contributed to the genetic variability. Secondly, differential seed longevity may be related to seed dormancy (Raatz et al. 2012). Although both populations had a similar degree of primary dormancy (Chapter 4), the secondary dormancy potential of these populations is unknown. Secondary dormancy potential of a species is often related to its seed bank persistence and influenced by environmental factors. The conditions of seed burial such as water stress and darkness may have enforced secondary dormancy in seeds under field conditions (Pekrun 1994). For example, *B. napus* seeds are non–dormant at maturity, but when buried, particularly under dry conditions, can enter secondary dormancy, which can

persist for longer periods (Lutman et al. 2003). The third reason could be due to differences in seed size.

The smaller seed size may have facilitated the easy burial of the weedy population, which may have resulted in a larger seed bank compared to the cultivated population. Seed size has been an important correlate to predict seed persistence under soil seed bank conditions, with smaller seeds often showing higher soil seed persistence than larger seeds (Peco et al. 2003). Since, the fate of a majority of cultivated seeds could not be explained by emergence or by persistence; the influence of other factors such as predation may have affected seed survival. A direct relationship between seed size and the effect of seed predation on seedling emergence has been proposed (Facelli and Pickett 1991). It is generally believed that predators can locate larger seeds more easily than smaller seeds. As a result, predation may reduce seed bank formation less for species with small seeds than with large seeds (Reader 1993). Furthermore, poor seed burial due to large seed size of the cultivated population may have retained more seeds on the soil surface allowing greater predation.

Tillage often affects seed persistence (Benech–Arnold et al. 2000; Gruber et al. 2004). In our study, spring–tilled treatments had a greater seedling emergence (Figure 5.2) and a smaller residual soil seed bank (Figure 5.3) than no–till regardless of the population at most locations (Table 5.5). In most cases, tillage improves emergence and reduces the chance of persistent seed bank formation by stimulating germination, whereas buried and no–till situations generally hamper germination (Froud–Williams et al. 1984). The micro–environmental factors such as light exposure, moisture availability, temperature fluctuations and predator activity have the potential to affect seed persistence under field conditions. It has been suggested that the lower seedling recruitment under the zero–tillage system may be due to lack of seed incorporation into soil and subsequent rapid desiccation (Mohler and Galford 1997). This was not the case in our study, although seeds were broadcasted initially, we observed a larger seed bank in no–till treatments (Figure 5.3). Hence it is unlikely that poor emergence in no–till treatments was due to lack of incorporation, but could be because of other factors such as darkness and minor temperature fluctuations, which were previously determined to be important factors for cow cockle primary dormancy and germination (Chapter 4).

Seedling emergence during the final year of seed burial (2011) was significantly different between the tillage treatments only at the Saskatoon location (Figure 5.2). Furthermore, at all locations, we observed a higher number of seedlings in zero–tilled treatments compared to spring tilled, suggesting a greater number of viable seeds available for germination. This is also reflected in the size of the residual (viable) seed bank (Figure 5.3). Similar results were observed by Gruber et al. (2004) in volunteer oilseed rape. They found a high autumn emergence in no–till treatments compared to stubble tillage treatments. We found no differences in the size of the soil seed bank between the populations under spring tillage (Figure 5.3), however in a no–till condition, a larger soil seed bank of the weedy population compared to the cultivated was observed (Figure 5.3). This may be due to greater seed predation in the cultivated population and high emergence of weedy population under spring tillage conditions. In contrast, under no till conditions, the extent of predation in the cultivated population may have been similar to that in the spring tillage treatment, but the reduced emergence in the weedy population may have contributed to the differences in residual seed bank. This suggests an overall greater seed persistence of the weedy population. Spring tillage has been observed to have a greater effect in reducing the seed bank of weedy population compared to a cultivated population. For example in velvetleaf, Lueschen et al. (1993) demonstrated a high seed survival of 15–25% after 17 years of seed burial in no–till treatments compared to mouldboard ploughed treatments, where only 0.8–2.5% of the initial seed was recovered. Similar results were also reported by Cardina et al. (2002).

Seed longevity is the inherent life span of seeds (Cavieres and Arroyo 2001). In the laboratory study, the seed longevity differences among the populations were considerable (Figure 5.5). Seed dormancy partially determines persistence in soil seed banks (Adugna 2013; Saatkamp et al. 2011). Furthermore, a positive relationship between seed longevity (laboratory) and seed dormancy has been suggested (Jurado and Flores 2005) but has not been established. In our study, we observed that the p_{50} values from the aging experiment seem to have been influenced by primary seed dormancy. Cow cockle exhibits conditional dormancy at maturity, a state at which seeds germinate over a narrower range of conditions compared to non–dormant. Mongolia, a completely non–dormant population survived for the least number of days (p_{50}) under controlled aging conditions. All the remaining populations have conditional dormancy however the degree of dormancy varied among the populations. Most of the weedy populations

germinated at a wider range of temperatures compared to cultivated and ornamental populations, suggesting a lower dormancy. These weedy populations also tended to have lower p_{50} compared to the cultivated and ornamental populations (Figure 5.5).

The geographic origin of the populations may have contributed to seed longevity, as species originating from cool, temperate climates tended to produce short–lived seeds (Walters et al. 2005). Among the weedy populations, UMan–89, Scott Weedy and Saskatoon Weedy had seed survival curves comparable with most cultivated populations. The similar seed survival in these two groups may be because of their similarities in dormancy and germination characteristics. Although the optimal germination temperature of Florist Rose and White Beauty was found to be < 10 C, they survived longer than the rest under aging conditions (45 C and 60% RH). Being ornamentals, high seed viability is selectively advantageous to avoid planting every year. Hence there could be a possible selection for greater viability in these populations. The correlations between seed longevity and germination traits indicate a genetic basis for the variation among populations originating from different eco–geographic regions.

Adopting the comparative classification by Long et al. (2008), we categorized our populations into different classes based on the laboratory controlled aging test. Mongolia (Group 1) with p_{50} values in the range of 20–25 days may develop transient seed banks with field persistence less than a year. All the weedy populations from Manitoba (Group 2) except UMan–89 are predicted to form short–lived seed banks (field persistence 1–3 years), as their seeds survived for 25–50 days in aging conditions. Remaining populations (Pink Beauty, Turkey, Scott [Cultivated population], PB–87, UMan–89, Scott Weedy [Weedy population] and Saskatoon Weedy) with p_{50} values >50 days corresponded with greater field persistence of over 3 years. The ornamental populations of Group 4 (Florist Rose and White Beauty) may persist even longer.

Long et al. (2008) observed a correlation between seed longevity estimated through comparative aging studies and field seed bank persistence in several European and Australian weed species. Our findings also suggested a partial link between field seed persistence and laboratory aging studies. In our study, we compared the cultivated population (Scott) with the weedy population (Scott Weedy) in both accelerated aging and field conditions. In the field study (seedling emergence and residual seed bank), although we observed lower seed persistence in the

cultivated compared to the weedy population, the cultivated population still showed substantial seed persistence suggesting a partial domestication. Additionally, the residual seed bank results also suggest that both these populations persist > 3 years. This concurs with the results of laboratory study, as both the populations had p_{50} values > 50 days which corresponds to field persistence of over three years. However, the difference between these two populations in the field seed persistence was not reflected in the aging study. We propose that seed characteristics such as dormancy and seed size which aid in seed burial may have caused seed longevity (laboratory) differences between the populations. Seed dormancy is an important factor in persistent seed bank formation (Thompson et al. 2003; Baskin and Baskin 2004). Similarities observed in this study between laboratory aging and the field experiment support the assumption that there is a genetic basis of seed persistence (Cavieres and Arroyo 2001; Momoh et al. 2002; Long et al. 2008). Although laboratory aging studies demonstrated significant differences in seed longevity among the populations, the environmental complexity of field conditions may involve several other factors to affect seed persistence.

Even though the cultivated population of cow cockle is less persistent than the weedy population under field conditions, there was still a considerable amount of seed persisting in the soil after 3 years. Because of this cow cockle volunteers may become problematic weeds in subsequent crops. Chadoeuf et al. (1998) reported lower persistence of cultivated *B. napus* compared to weedy hoary mustard, but also observed that more than 1% of cultivated rape seed survived after 3 years under field conditions. Similarly, in our field study, there is still a substantial amount of un–germinated seed remaining for both populations after the first growing season and with the observed seed persistence level; we believe that these remaining viable seeds may form a persistent soil seed bank.

The seed physical and physiological characteristics that are developed to adapt their native or non–native habitats may have contributed to the seed longevity differences in these populations. The study identified substantial seed persistence in cow cockle; this may pose some concerns for the production of cow cockle as a crop for the Canadian prairies. Agronomic practices may have to be optimized to tackle the seed persistence problems in cow cockle; such as harvesting to reduce the combine losses and pre–seed spring tillage followed by in–crop herbicide control. However the ultimate solution would be for plant breeders to select for

varieties without seed dormancy and reduced seed bank persistence. Similar results for both field and laboratory studies suggest that the artificial aging test used in this study may be viable test for plant breeders to use when selecting against seed longevity.

6.0 GENERAL DISCUSSION

The primary hypothesis of this research was that different populations of cow cockle will exhibit varying degrees of weediness. This hypothesis was tested by investigating the domestication status of this species. Therefore, three studies were conducted to investigate different components of domestication syndrome including phenotypic modifications, seed dormancy and seed persistence.

6.1 AGRONOMIC AND MORPHOLOGICAL VARIABILITY

Evaluation of agronomic and morphological variability is a simple and cost–effective method to study the phylogenetic history of a plant species. The primary objective of the agronomic and morphological study was to evaluate available cow cockle germplasm to determine the populations that are best adapted to the cultivation as well as the traits responsible to such adaptation. Results presented in this thesis suggest that Pink Beauty, Turkey, PB–87, Scott and UMan–89 are most suitable for cultivation. These populations showed characteristics of domesticated crops including synchronous growth habit, greater thousand seed weight and seed yield (Chapter 3). Among the populations, Pink Beauty, Turkey and PB–87 are cultivated populations; Scott is a semi–domesticated population and UMan–89 is a weedy population, which appeared to have pre–adapted to domestication. According to the recent protracted domestication model, the domestication of crop plants is initiated with prolonged pre–domestication cultivation (Willcox et al. 2009; Allaby 2010). Based on the findings of this research, I believe that similar selection pressures may have resulted in these pre–adapted or partially domesticated populations. However, due to the limited knowledge regarding the phenological history of these populations, we are unaware of the time frame these selection pressures may have been occurring to attain such adaptability. Gepts (2004) also supports the idea that morphological domestication was preceded by a stage of cultivation. The weedy populations in the study (UMan–00, 02, 04, 05, 88, Scott Weedy and Saskatoon Weedy) also showed signs of adaptability to cultivation such as uniform germination, flowering and maturity (Table 3.4). According to Harlan and De Wet (1965), a process of adaptive trait selection similar to that of crop plants, also resulted in weed species. However, unlike domesticates, weedy populations are capable of surviving in man–made habitats without any requirement of artificial

propagation (De Wet and Harlan 1975). Despite being adapted to cultivation, the weedy populations in the study possess characteristics that may help in self–perpetuation.

The plant characters physiological maturity, seed size, plant height, flower size and seed yield differed most in cow cockle populations (Table 3.2), suggesting their role in the process of adaptation to different selection forces. Although flower size showed greater variability, the adaptive significance of this trait is unclear, since both wild and weedy populations produced similar–sized flowers. The pre–adapted populations (Pink Beauty, Turkey, PB–87, Scott and UMan–89) were characterized by tall plants, medium maturing, large seeds and high seed yields (Figure 3.5, 3.3, 3.4 and 3.6). Crop cultivation regime offers a different set of conditions when compared to natural habitats, where in which, certain traits such as gigantism, vigor are selected and in contrast, selection occurs against traits relating to self–dependency (Ladizinsky 1998). Cultivation increases intra–specific competition due to increased plant density, which often selects for high seedling vigor which produces larger individuals (Harlan et al. 1973). Increased seedling vigor is frequently associated with an increase in seed size. Increase in seed size is generally related to a decrease in the seed yield of the individual plant. However, overall crop yield must have increased under cultivation due to increased tolerable plant density per unit area (De Wet and Harlan 1975).

Similar to cultivated populations, the weedy lines evaluated exhibited characters such as early vigor, synchronized growth stages, intermediate seed yield and phenological adaptation to new environments. Furthermore, they also retained their self–sustaining characteristics such as short plant height with bushy architecture that aid in tumbleweed seed dispersal; early maturity to escape weed control measures; and production of small seeds as a function of seed burial and persistence suggest weediness potential in these populations. Therefore, it is possible to believe that the selection for a weedy habit prepared these Canadian populations for an initial stage of domestication (Chapter 3).

6.2 SEED DORMANCY IN COW COCKLE

The objective of this study was to examine the available cow cockle germplasm for primary seed dormancy and determine how temperature and light affect seed dormancy and germination. Cow cockle exhibits a high degree of primary dormancy at harvest (Chapter 4 and

Figure 4.1). Primary seed dormancy is generally genotypic and often reduced or eliminated during the process of domestication (Harlan et al. 1973). Although high seed dormancy is undesirable in domesticates, a complete elimination of it may result in problems such as pre–harvest sprouting especially under cold and humid environments (Gubler et al. 2005). In cow cockle, the apex of the capsule opens into a four–toothed orifice at maturity exposing the seeds to external environmental conditions before the dispersal of the seed (Crandall 1893). Thus I believe that a certain degree of primary dormancy at harvest is a desired trait as it prevents early germination of seeds in the capsule, following exposure to cool moist conditions.

The preferential germination of cow cockle at the specific temperature of 10 C suggests a conditional type of dormancy (Figure 4.1). Conditional dormancy is a transitional stage between dormancy and non–dormancy (Baskin and Baskin 1998). Based on the germination response patterns, the cow cockle populations were categorized into groups similar to those of the agronomic and morphological studies (compare Figure 3.1 and 4.1). The cultivated populations with large seeds, greater yield and longer maturity period have shown germination patterns different from other groups. Similarly, the small–seeded and early maturing weedy populations showed characteristic germination curves which varied from those of the ornamentals and wild populations. All the populations were found to be conditionally dormant at maturity except Mongolia. However, I found some variability in the preferential germination temperature among cow cockle populations. Since these populations originated from different parts of world, the discrepancies in soil, water, light, temperature and other environmental conditions may have possibly contributed to their variable germination strategies. A possible selection pressure of the species habitat conditions on their germination strategies (Baskin and Baskin 1988). Mongolia (PI 597629) was completely non–dormant, which had originally been collected from a desert–steppe ecological zone (Sasaki et al. 2008). In deserts, moisture necessary for germination and seedling establishment is only transiently available after precipitation; presence of seed dormancy in such habitats may limit the chances of germination and successful seedling establishment. Thus, this population's non–dormancy germination strategy likely evolved in response to the conditions in its geographical region.

The results of this thesis indicate both temperature and light control germination of conditionally dormant cow cockle seed (Chapter 4). Furthermore, with optimal temperature

conditions, the effect of temperature regime (alternating and constant) and light were insignificant (Figure 4.1). At sub–optimal and supra–optimal conditions, alternating temperatures had a major impact on the germination of conditionally–dormant cow cockle seed, followed by light. Additionally, with these conditions, the requirement of alternating temperatures to promote seed germination could be partially replaced by light in cow cockle. Ecologically, both alternating temperatures and light help in sensing the depth of burial and that prevents fatal germination from deeper layers (Schutz et al. 2002). My findings also suggest a greater requirement of light for seed germination especially in weedy populations. This germination response may be related to seed size, as the weedy populations have smaller seeds compared to the other populations. Small seeds with nutrient supplies inadequate for germination from greater depths use light as a soil–depth indicator that helps in germination and seedling recruitment. These results are supported by other researchers, who have observed an inverse relationship between seed mass and requirement of light (Milberg et al. 2000; Aud and Ferraz 2012). Under natural conditions, the requirement of light or alternating temperatures suggests that even dormancy–broken seeds can form a soil seed bank, associated with low temperature inhibition in winter, to withstand adverse climatic conditions. Based on the findings of this research, I believe that small–seeded and conditionally dormant weedy populations in the study have higher chances of forming a persistent seed bank under unfavorable environmental conditions because of their ease of burial coupled with germination inhibition under winter conditions.

Domestication of many seed–propagated species has included selection for sufficient primary dormancy to prevent immediate germination after harvest. Therefore the conditional dormancy in cow cockle can be observed as an evolutionary mechanism that prevents untimely germination following maturity and may not be considered as a major obstacle for its domestication.

6.3 SEED PERSISTENCE

The objective of the study involves evaluation of available germplasm to determine if cow cockle is persistent under conditions of cultivation and forms a seed bank. Two populations including weedy (Scott weedy) and cultivated (Scott) lines were included in field study. The results from the 3–year field study showed consistently higher emergence of the weedy

population than the cultivated population (Figure 5.1). In addition to a higher emergence, a substantially higher numbers of seed were recovered from the soil for weedy population compared to the cultivated population for all the locations; which suggests greater seed persistence of the weedy line (Figure 5.3). Most weed species develop persistent seed banks (Holm et al. 1997) when compared to cultivated species , which are less persistent or develop transient seed banks. For example, Chadoeuf et al. (1998) reported a slower rate of viability loss in weedy hoary mustard (*Hirschfeldia incana* L.) compared to cultivated rape (*Brassica napus* L.) and inter–generic hybrid between rape and hoary mustard. The high proportion of unrecorded losses in cultivated population suggests the influence of factors such as predation and fungal attack affecting the seed survival, which were not measured in the present study. Although several physical and physiological factors that influence seed survival in soil, it is often believed that, the inherent capacity of un–germinated seed to resist breakdown of the seed coat by microbes and predators is more important to build a persistent seed bank. Another possible explanation for the seed persistence variability would be seed size. The ease of burial and predator avoidance of small–seeded weedy population may have contributed to the higher seed persistence compared to the cultivated line. The seed burial hypothesis in relation to seed persistence has been illustrated in many species in a wide range of habitats (Cerabolini et al. 2003; Peco et al. 2003). Despite the differences in seed persistence between the populations, considerable numbers of seed of both weedy and cultivated lines were recovered from the soil seed bank at the end of the study. This depicts that cow cockle is persistent and can form a reasonably long–term seed bank.

The greater emergence (Figure 5.2) and smaller residual soil seed bank (Figure 5.3) in tilled plots suggests that spring tillage has a diminishing effect on the soil seed bank by promoting seed germination. Conversely, zero tillage increases the chances of persistent seed bank formation in most cases. I found no differences in the size of the soil seed bank between the populations under spring tillage (Figure 5.3), however under no till conditions, a larger soil seed bank of the weedy population compared to the cultivated population was observed at the end of the study (Figure 5.3). This may be because of greater seed predation in the cultivated Scott population and high emergence of the Scott Weedy population in the spring tillage plots. In contrast, under no till conditions, the extent of predation in the cultivated population may have been similar to spring tilled, but the poor emergence in the weedy population may have

contributed to the differences in residual seed bank. This suggests a greater seed persistence of Scott Weedy population, additionally, a predominant effect of spring tillage on the soil seed bank of this population compared to the Scott line. Similar results were reported by Froud–Williams et al. (1984) in their study on sixteen arable weeds, as they found a greater number of seeds remaining after the 2 year burial when seeds were buried and undisturbed. Cardina et al. (2002) also reported a high weed seed persistence in no–till compared to till treatments.

All 15 populations were included in the laboratory study of seed persistence. Unlike the field study, seed size did not explain differences in seed longevity among the populations under artificial aging conditions (45 C and 60% RH); as most of the small–seeded weedy populations had low p_{50} values when compared to large–seeded cultivated populations (Table 5.3). Furthermore, primary seed dormancy seems to have influenced the seed survival under aging conditions (Table 5.3). Seed of Mongolia, a completely non–dormant population, survived for the least number of days (p_{50}) under aging conditions. Additionally, Florist Rose and White Beauty, which were among the highly dormant populations, also survived longer than the other populations (Figure 5.4). A relationship between dormancy and seed longevity has been proposed (Jurado and Flores 2005).

Both field seed persistence and laboratory studies showed similar results in relation to the seed persistence of cultivated and weedy populations (Chapter 5). Although I observed significant seed persistence differences between the populations under field conditions, the residual seed bank results suggest that both the populations persist > 3 years. This is in accordance with the results of the laboratory study, as both the populations had p_{50} values > 50 days which predicts a field persistence of over three years. These findings also suggest that even though the cultivated population is less persistent than the weedy population under field conditions, still a considerable amount of seed persists in the soil after 3 years and may pose volunteer problems in subsequent crops. Chadoeuf et al. (1998) reported the survival of more than 1% of cultivated rape seed after 3 years under field conditions may pose a serious problem in relation to the huge seed loss at harvest. A supporting explanation for substantial seed persistence in cultivated population might be because of its secondary dormancy potential. Unfavorable environmental factors such as water stress and darkness may have induced secondary dormancy in seeds under field conditions (Pekrun 1994).

Overall, the three studies in this thesis attempted to understand the domestication status of cow cockle. The agronomic adaptations such as higher seed yield, larger seeds, and greater biomass in Pink Beauty, Turkey, PB–87, Scott and UMan–89 suggest a partial morphological domestication. The agronomic characteristics of UMan–00, 02, 04, 05, 88, Scott Weedy and Saskatoon Weedy indicated that these populations may have evolved to adapt the recurrent disturbances in agricultural systems; however still possess characteristics that support weedy behavior. Additionally, the non–deep conditional dormancy in cow cockle may not be considered as a barrier for its domestication and can be viewed as a physiological mechanism to avoid germination at harvest. The major concern in cow cockle domestication would be seed persistence, as significant amount of seed survived in the field even after three years. These findings led to acceptance of the main hypothesis; as most of the cow cockle populations from Canada are weedy with some adaptation to cultivation, suggesting a stage of pre–domestication.

6.4 FUTURE RESEARCH

This research has provided important information on domestication status of cow cockle in aspects of adaptation to cultivation, primary seed dormancy and seed persistence. The study identified that there are no strong obstacles for its domestication except some concerns in seed persistence, which have to be addressed before it is grown as a crop. Although the field emergence is greater than 50% in most populations, still a substantial amount remained in the soil un–germinated; and with given seed persistence level, the viable seeds in the soil left from seeding along with that lost at harvest may pose volunteer problem in subsequent crops. Secondary dormancy potential of a species is often related to its seed bank persistence. Apart from some preliminary laboratory studies (data not shown), there has been no conscious research on secondary dormancy potential in cow cockle. A laboratory study in which artificial induction of secondary dormancy using polyethylene glycol (PEG–8000) under dark conditions for 4 weeks followed by germination and viability tests would be informative. In my persistence study, only two populations belonging to similar dormancy group were included. Although I observed differences in seed persistence under field conditions, the factors affecting the seed persistence were not measured. Seed burial study involving more number of populations with diverse physical and physiological characteristics would add to our understanding of seed bank ecology of cow cockle.

Tumbleweed seed dispersal has been reported in early introduced populations of cow cockle (Crandall 1893). High seed persistence coupled with wild mode of seed dispersal favors weediness in agro–ecosystems and invasiveness in natural conditions. In the field experiment, I quantified seed shattering to some extent but a more comprehensive study to measure the harvest losses and to examine the mode of dispersal among different populations is needed. In a field study, harvest losses can be quantified using catch trays in each plot and monitoring the seed loss from mid–seed filling stage to harvest. Some of the other issues I observed under field conditions were in–crop broadleaf weed control and alternaria leaf spot. Cow cockle can tolerate clethodim and isoxaflutole herbicides (Efthimiadou et al. 2012), which are not effective against in–crop broadleaf weeds. The crop was also observed to be susceptible to leaf spot caused by *Alternaria saponariae* (Peck) Neergaard. It infects the plant at the late flowering stage to early pod stage causing serious yield losses. Resistant varieties or fungicidal options are required before commercial production can proceed.

Ideally, from the standpoint of a plant breeder, the lack of primary seed dormancy in the population Mongolia, greater agronomic potential especially yield of cultivated or semi–cultivated populations are of prime importance. Further breeding efforts should be directed to optimize the use of genetic variation in these populations by bringing together all the desirable characters in one genotype that maximize yield and reduce environmental or management risks. Since seed compositions are important in cow cockle, in order to realize full crop potential, efficient large–scale processing and extraction methods need to be developed with supporting markets for different seed constituents.

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APPENDICES

Appendix 1. Emergence, Floral Initiation, Number of Branches and Shoot Biomass of cow cockle (*Vaccaria hispanica* [P. Mill.] Rauschert) populations assessed at Saskatoon and Edmonton locations.

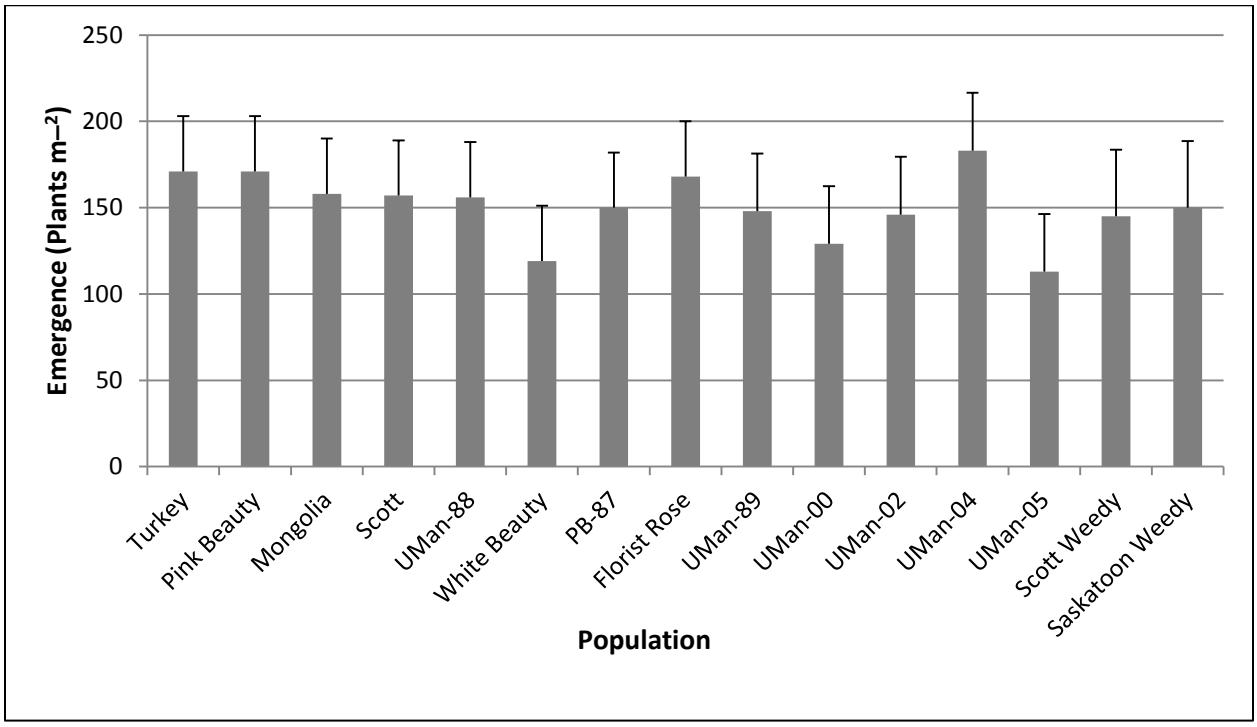

Figure A1.1 Emergence of cow cockle populations assessed at Saskatoon (2009, 2010 and 2011) and Edmonton (2009). Error bars represent the standard errors of least squares.

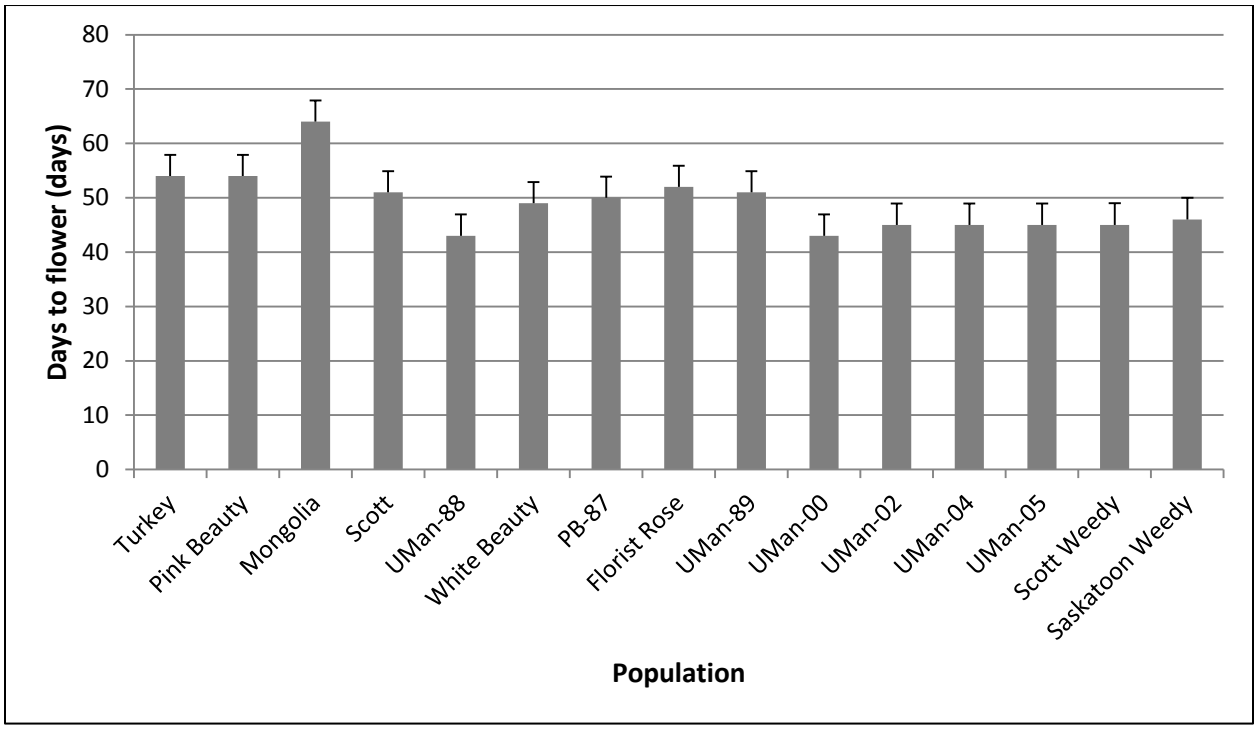

Figure A1.2 Floral Initiation of cow cockle populations assessed at Saskatoon (2009, 2010 and 2011). Error bars represent the standard errors of least squares.

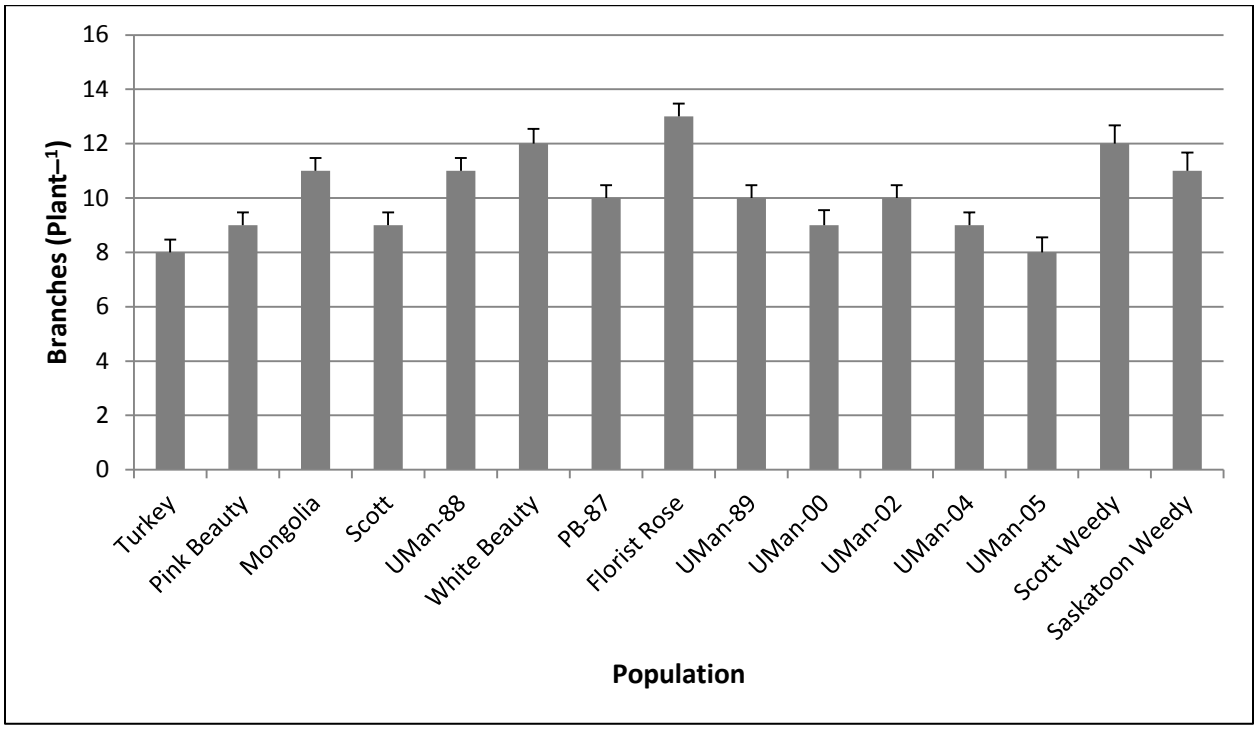

Figure A1.3 Number of branches of cow cockle populations assessed at Saskatoon (2009, 2010 and 2011). Error bars represent the standard errors of least squares.

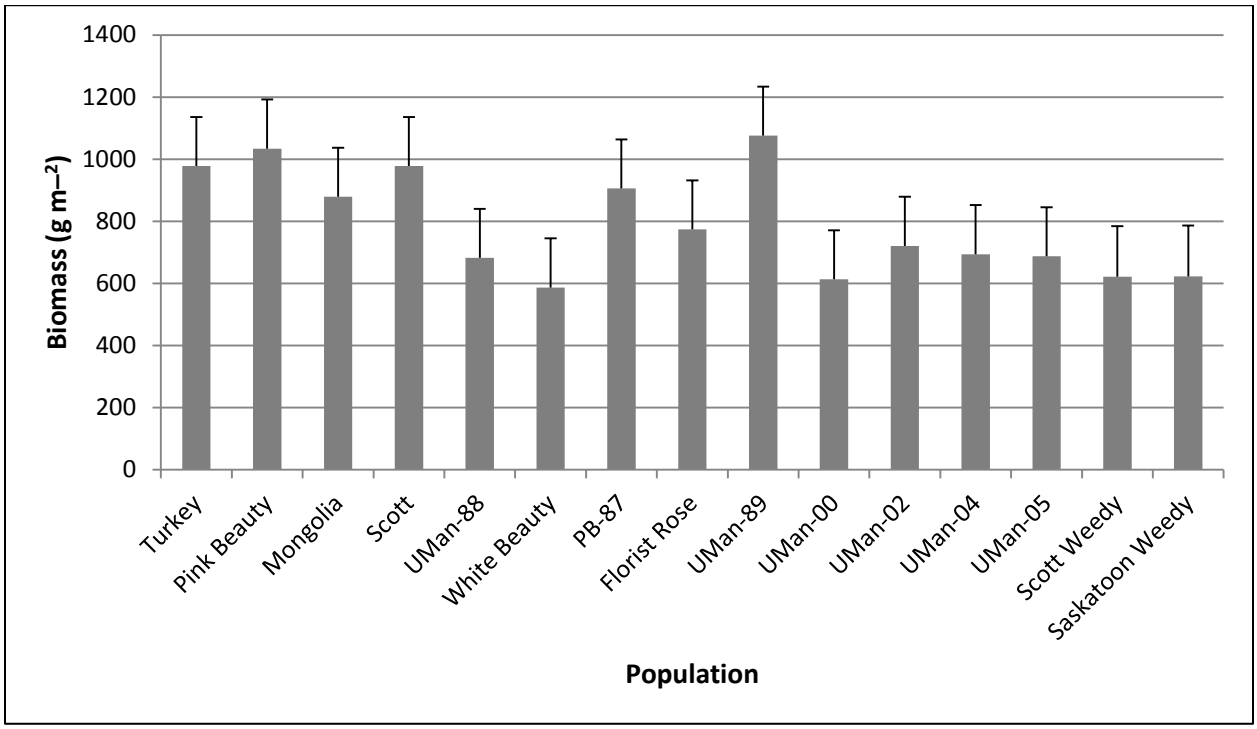

Figure A1.4 Biomass of cow cockle populations assessed at Saskatoon (2009, 2010 and 2011). Error bars represent the standard errors of least squares.

Appendix 2. Seed germination response to temperature and light of 15 cow cockle (*Vaccaria hispanica* [P. Mill.] Rauschert) populations.

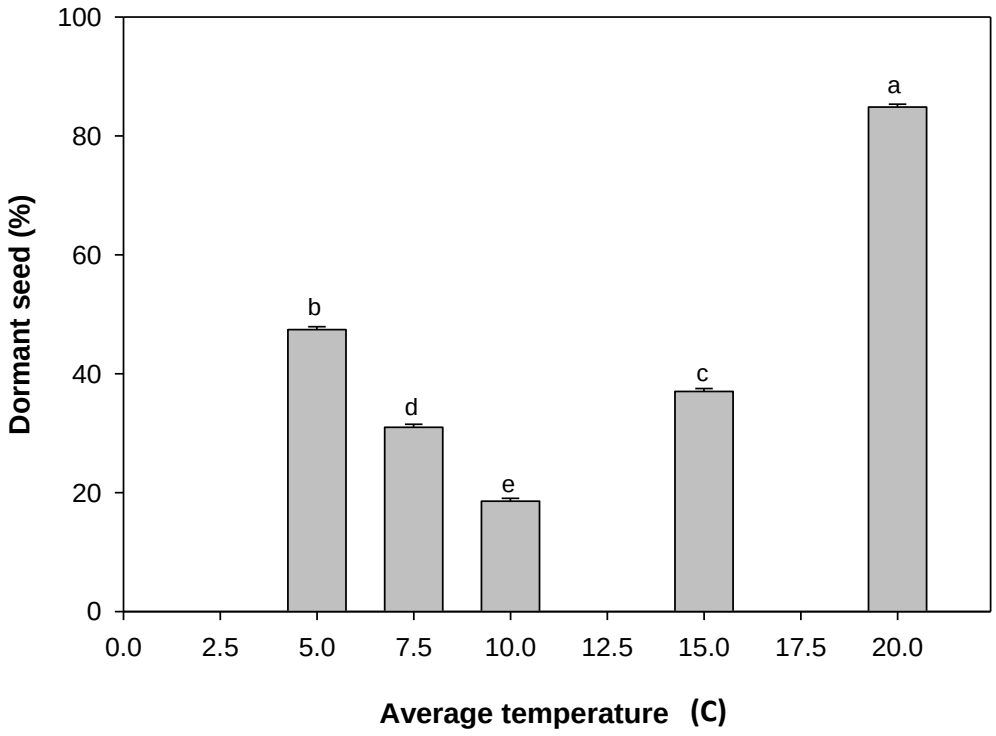

Figure A2.1 Effect of mean temperature on seed dormancy. Error bars represent the standard errors of least squares. Comparisons are made among the temperatures, with similar letters indicating no significance at $LSD_{0.05.}$

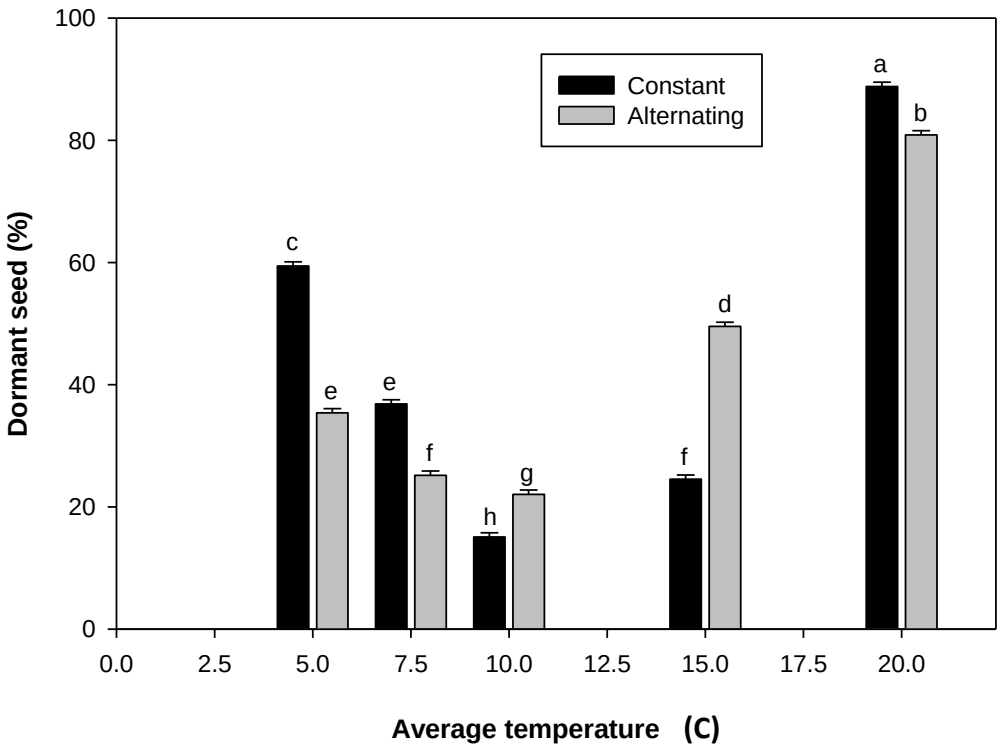

Figure A2.2 Effect of mean temperature under different regimes on cow cockle seed dormancy. Error bars represent the standard errors of least squares. Comparisons are made between the temperature regimes, with similar letters indicating no significance at $LSD_{0.05.}$

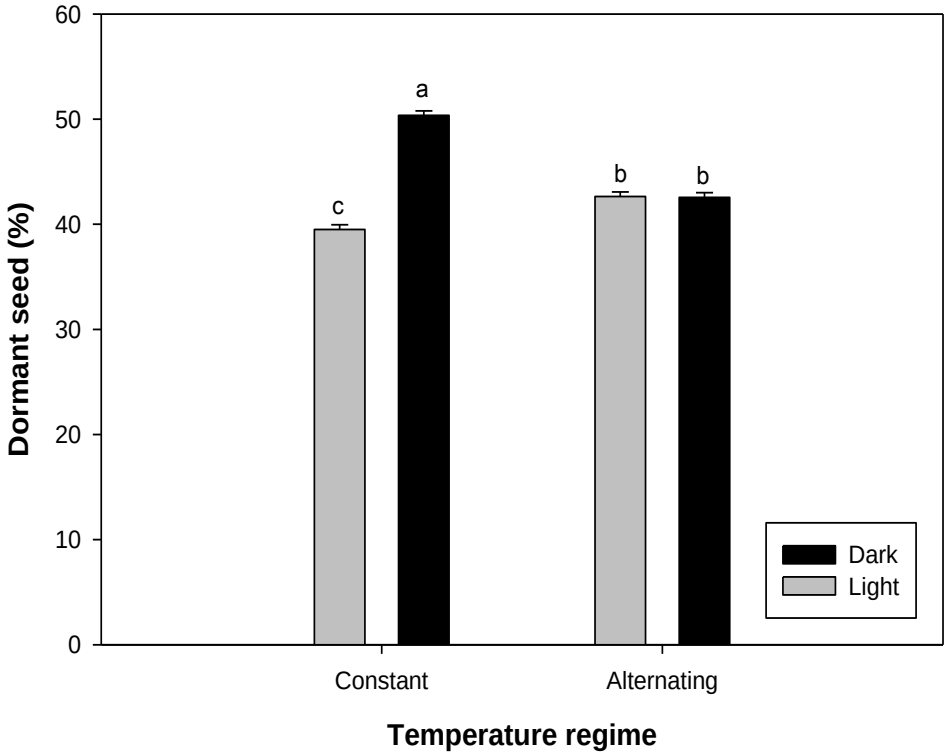

Figure A2.3 Effect of light under different temperature regimes on seed dormancy. Error bars represent the standard errors of least squares. Comparisons are made between dark and light, with similar letters indicating no significance at $LSD_{0.05.}$

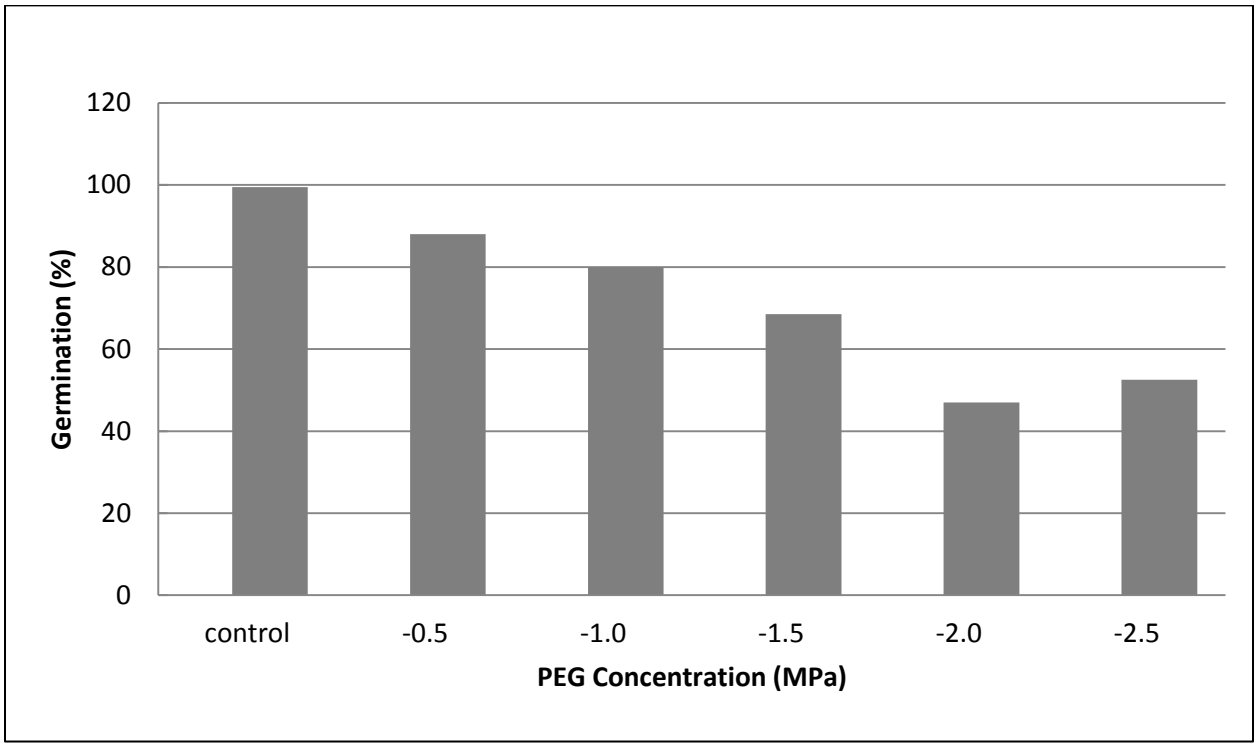

Figure A2.4 Secondary dormancy potential of a weedy cow cockle population at different concentrations of PEG (Polyethylene glycol).